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JOURNAL OF DAIRY SCIENCE

VOLUME XX NOVEMBER, 1937 NUMBER 11

THE ACCURACY OF THE DIRECT MICROSCOPIC (BREED) COUNT OF BACTERIA AND LEUCOCYTES IN MILK

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INTRODUCTION

For many years the dairy bacteriologist has been attempting to evaluate the methods in use for the quantitative estimation of bacterial populations in milk. To this end a considerable body of data is now available showing the variability of the plate count and to some extent the relation of the plate count to other methods of analysis. Notwithstanding this, it is not yet possible to estimate with reasonable precision the accuracy of any present method.

Although it is frequently conceded that, except when applied to milk of very low bacterial content, the direct microscopic or Breed count offers the maximum accuracy, no such body of data relative to the accuracy of this test as in the case of the plate count has appeared (2, 3, 4). The present discussion is, therefore, not untimely.

Because of the recent remarkable advances in the technique of sanitary milk production market milks are tending more and more to approach milks aseptically drawn. Any method to be intelligently used for the enumeration of the bacteria in raw market milk should be viewed in the light of this improvement. Therefore, in the present study only milks drawn aseptically from the udder were placed under observation. It is a general belief that the Breed count is least accurate when applied to this type of milk and the inaccuracies are assumed to be due to:

1. The non-representativeness of the small portion of milk examined.
2. The failure of some bacteria to stain.
3. The non-recognition of stained particles as bacteria or foreign matter.
4. The personal factor.

Received for publication July 19, 1937.

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The data herein contained are taken from a thesis presented at the University of Alberta by N. J. Strynadka in partial fulfilment of the requirements for the degree of Master of Science. The work was supported in part by a grant from dairy interests within the province.

Some of these sources of inaccuracy are minimized by extending the microscopic search and this was done in the present study.

METHODS

Samples of milk were obtained by carefully drawing into sterile flasks or test tubes an approximately equal amount of milk from each half-empty udder which had previously been wiped with a chlorine solution. The maximum time elapsing between the drawing of the milk and the preparation of the smears was three hours, the usual time being not over one hour and a half.

Standard Methods of Milk Analysis (1) was followed and the Breed factor for the microscope was almost exactly 600,000. For the sake of accuracy a hand tally was used in counting which was done within a circular eye-piece micrometer delineating the central flat portion of the field. Nowithstanding this, considerable focusing was found necessary for the proper identification of many bacterial cells. Care was taken to minimize the overlapping of fields. The preparation of the smears and the counting was all done by one worker (S) and all counts are reported on a per cc. basis. All cells other than those of microorganisms are classed as leucocytes and the possibility of the presence of unstained and, therefore, uncountable leucocytes and bacteria in the milk is disregarded.

RESULTS

The Accuracy of the Microscopic Count of Bacteria in Milk

In 9 (40.4 per cent) of the 22 milks reported in Table 1 no bacteria were observed in the first 60 fields examined, while in 4 (18.1 per cent) over 100 fields were examined before any bacteria were found. The counts based on the examination of 60 fields were larger than the counts based on 1000 fields in the case of 4 (18.1 per cent) miks. The counts based on 1000 fields were greater than the counts based on 2000 fields in the case of 1 (11.1 per cent) of 9 milks. There is no tendency for these exceptions to occur only in milks containing bacteria in large clumps. Two possible explanations are the failure to recognize bacteria occurring singly and the uneven distribution of such organisms.

No bacteria were observed in the first 60 fields of any milk having a 1000 field count of 35,000 or less. This, of course, is due to chance but the chance undoubtedly approaches certainty as the bacterial content decreases. It does not necessarily follow that the 2000 field counts are the most accurate but they are probably so in most cases because the influence of the exceptional clump is lessened as the number of examined fields decreases.

It is apparent from these data that bacteria are very unevenly distributed in this class of milk even when clumping is not extensive. So true is this that the 60 field counts were usually misleading and the 1000 field counts frequently so.

TABLE 1

Breed counts of 22 milks based on the examination of 60, 1000 and 2000 fields per smear

MILK NO.	BREED COUNTS			FIRST FIELD SHOWING BACTERIA	LARGEST GROUP WAS FOUND IN FIELD NO.	CELLS IN LARGEST GROUP
	60 fields	1000 fields	2000 fields			
1	80,000	363,000		11	814	232
2	10,000	35,000		83	832	8
3	10,000	107,400		75	75	97
4	10,000	50,400		69	967	6
5	40,000	121,200		4	87	60
6	10,000	9,000		110	929	2
7	10,000	99,600		31	886	36
8	10,000	7,200		189	882	2
9	10,000	12,000		69	253	10
10	10,000	15,600		120	120	6
11	10,000	18,000		115	312	2
12	90,000	158,400		18	104	150
13	10,000	7,800		98	281	2
14	10,000	39,600	139,800	57	1252	148
15	370,000	48,600	34,800	47	47	14
16	30,000	64,800	147,800	30	1620	250
17	90,000	63,600	119,700	16	1114	34
18	90,000	81,600	200,700	29	1754	350
19	170,000	115,200	477,000	3	1093	200
20	70,000	138,000	142,400	32	65	24
21	60,000	120,000	146,400	57	1050	58
22	20,000	64,000	132,800	30	1267	150

To further study the uneven distribution of bacteria in milk and its effect on the Breed count 8 replicate smears were prepared from each of 2 milks. During the preparation of these smears the milk was gently agitated and the same pipette was used throughout, being thoroughly cleaned and

TABLE 2

The 6000-field (entire smear) bacterial counts of 2 milks, 8 replicate smears of each milk being examined

SMEAR NUMBER	MILK 23			MILK 24		
	Largest group	Group counts	Individual counts	Largest group	Group counts	Individual counts
1	2 cells	8,400	9,300	28 cells	17,900	25,400
2	16 cells	10,800	13,200	18 cells	29,000	38,500
3	200 cells	9,200	30,600	50 cells	23,400	53,500
4	22 cells	9,200	12,900	8 cells	21,600	28,600
5	60 cells	8,400	17,600	6 cells	32,600	38,600
6	4 cells	10,600	12,600	50 cells	41,500	60,100
7	4 cells	8,800	12,800	6 cells	37,300	44,800
8	10 cells	8,100	10,400	10 cells	42,200	46,400
Mean		9,187.5	14,925		30,687.5	41,987.5
Percentage maximum deviation from the mean		17.55	105.02		41.63	43.11
Ratio lowest to highest count		1: 1.33	1: 3.29		1: 2.35	1: 2.36
Average percentage absolute deviation from the mean		6.93	30.73		25.13	21.93

dried after each discharge. Each smear was examined in its entirety. Thus, each represents 6000 microscopic fields and, therefore, a total of 48,000 fields per milk was observed. From the results, which are presented in Table 2, it will be observed that the average absolute deviation from the mean of the group count is only 6.93 per cent for the first milk while it is 25.13 per cent for the second. On the other hand the same constants for the individual counts are 30.73 per cent and 21.93 per cent respectively. The uniformity of the group counts in milk 23 is remarkable.

Breed and Brew (2) reported 2 per cent as the maximum deviation from the mean for the accuracy of the Breed pipette in measuring samples of milk for the Breed smear, weight of the delivered sample being the criterion of accuracy. The variability shown in Table 2 is, therefore, likely to be due in the main to the uneven distribution of the bacteria in the milk. It is apparent that 0.01 cc. of this class of milk cannot be depended upon as being representative enough to secure a high degree of accuracy, so unevenly are the bacteria distributed.

The Accuracy of the Leucocyte Count

Prescott and Breed (4) made 31 leucocyte counts in duplicate, each count being based on the leucocytes observed in 100 microscopic fields, and found an average variation of 14.5 per cent. The variations in 2 milks containing less than 250,000 leucocytes per cc. were 42.9 per cent and 64.3 per cent.

It is to be expected that the leucocyte count is more accurate than the

TABLE 3

The 60-field leucocyte counts of 4 milks, a number of replicate smears of each milk being examined

SMEAR NUMBER	MILK 25	MILK 26	MILK 27	MILK 28
1	3,210,000	880,000	140,000	890,000
2	3,270,000	1,000,000	130,000	620,000
3	2,250,000	950,000	120,000	710,000
4	2,570,000	910,000	160,000	800,000
5	3,690,000	770,000	100,000	680,000
6	3,335,000	890,000	160,000	540,000
7	3,160,000	760,000	170,000	710,000
8	2,520,000	600,000	130,000	580,000
9	3,140,000	1,050,000	150,000	480,000
10	2,360.000		120,000	750,000
11			110,000	470,000
12			130,000	450,000
13				550,000
Mean	2,950,500	867,888	135,000	626,923
Percentage maximum deviation from the mean	25.05	30.85	25.70	42.07
Ratio lowest to highest count	1: 1.64	1: 1.75	1: 1.70	1: 1.97
Average percentage absolute deviation from the mean	13.90	12.11	12.96	17.14

TABLE 4

The 60-field leucocyte counts of 7 milks, a total of 720 fields of each smear being examined

COUNT NUMBER	MILK 29	MILK 30	MILK 31	MILK 32	MILK 33	MILK 34	MILK 35
1	580,000	4,230,000	370,000	100,000	4,730,000	740,000	1,560,000
2	1,130,000	3,070,000	220,000	120,000	5,810,000	750,000	1,730,000
3	1,270,000	3,100,000	340,000	60,000	5,780,000	740,000	1,900,000
4	1,290,000	3,430,000	390,000	50,000	6,590,000	1,030,000	2,120,000
5	1,510,000	4,010,000	310,000	80,000	4,910,000	810,000	2,150,000
6	1,360,000	4,630,000	280,000	130,000	5,780,000	1,060,000	1,850,000
7	1,430,000	3,980,000	300,000	90,000	5,090,000	700,000	1,700,000
8	650,000	3,390,000	250,000	50,000	4,910,000	780,000	1,580,000
9	1,160,000	4,170,000	430,000	60,000	4,440,000	540,000	1,480,000
10	1,480,000	4,260,000	340,000	50,000	4,080,000	770,000	1,600,000
11	1,600,000	4,060,000	300,000	60,000	4,600,000	730,000	1,830,000
12	1,400,000	3,820,000	340,000	80,000	4,700,000	770,000	1,790,000
Mean	1,238,333	3,845,850	322,500	77,500	5,116,666	783,333	1,774,166
Percentage maximum deviation from the mean	53.17	22.80	33.33	67.74	28.54	35.32	21.18
Ratio lowest to highest count	1: 2.75	1: 1.50	1: 1.95	1: 2.60	1: 1.61	1: 1.98	1: 1.45
Average percentage absolute deviation from the mean	19.29	10.29	14.21	29.03	11.34	11.48	9.34

Breed count of bacteria since leucocytes are larger and more easily recognized than bacteria.

In Table 3 the leucocyte counts of 4 milks are computed from the examination of 60 fields of each of a number of replicate smears. The maximum deviation from the mean ranges from approximately 25 per cent to approximately 42 per cent. The average absolute deviation from the mean is approximately 14 per cent, ranging from 12.11 per cent to 17.14 per cent, and the ratios of the lowest to the highest counts range from 1 : 1.64 to 1 : 1.97.

The question arises whether this variability is caused by the non-representativeness of the smear itself or of the small area of each smear which came under observation. This question is answered by a study of Table 4 in which 720 fields of each of 7 milks were examined and leucocyte counts computed for each 60 fields in the order of examination. The maximum deviation from the mean is seen to range from approximately 21 per cent to approximately 68 per cent and the average absolute deviation from the mean from 9.34 per cent to 29.03 per cent, averaging approximately 15 per cent. The ratios of the lowest to the highest counts range from 1 : 1.45 to 1 : 2.75. Therefore, the variability of the leucocyte count is due in the main to the uneven distribution of the cells in the smear while the smear itself is reasonably representative. There is no consistent tendency for greater variability of count as the leucocyte content decreases.

The extreme ratio of the lowest to the highest count is 1 : 2.75, while the average for the 11 milks is 1 : 1.9. If these figures are representative for usual milk supplies, the 60 field leucocyte count can be depended upon on the average to vary not more than $\pm$ 50 per cent from the mean and rarely will the highest count be greater than 3 times the lowest count of replicate samples. On the basis of these data we judge the 60 field leucocyte count to be much more accurate than the Breed count of bacteria in good milk unless the latter is based on the examination of more microscopic fields than is possible in practice.

DISCUSSION

Attempts to determine the accuracy of methods of counting bacteria in milk have included studies of variability and comparisons with other quantitative measures. Variability is not necessarily a true or adequate measure of accuracy. Comparison with another criterion is of limited value unless the criterion itself is accurately evaluated. The most accurate criterion is assumed by some to be the direct microscopic count if a representative portion of milk is examined. If this is true, there is no adequate criterion for determining the accuracy of the Breed count even when it is extensive enough to assure examination of representative portions of the milk. In an investigation of this test a study of variability, despite its inadequacy, seems to be the only available method of approach.

Forty-eight thousand microscopic fields of each of two milks were examined and the results reported in Table 2. To the best of our knowledge this is the most extensive microscopic search to which any milk to date has been subjected. It is thought that the results with these two milks serve as a check on the uniformity with which bacteria were recognized and identified in this study. There is probably little difficulty in identifying with certainty bacterial clumps, while organisms occurring singly are more likely to be overlooked. When a few fields are counted there may be a tendency, of which the microscopist may or may not be conscious, toward duplication of counts because of recollection of numbers. There is evidence in the literature for this being an important factor. When 6000 fields per smear are counted and the numbers recorded mechanically rather than mentally, this tendency toward automatic duplication would appear to be minimized. Therefore, the reported uniformity of dispersion of groups in milk 23 and lack of like uniformity in milk 24 probably approaches the actual condition of the bacteria in these milks. The writers see no reason for supposing that the personal factor differed in the examination of these two milks and it is possible that the reported lack of uniformity in the counts of individuals in each milk and in the group counts of milk 24 is evidence of considerable accuracy in the identification and counting in this study. The exact counting of the bacteria in a large clump is, of course, difficult. It is believed that the maximum inaccuracy introduced by this factor in the present study is not greater than 100 per cent.

Working with aseptically-drawn milk it was amply demonstrated that the milk observed in the examination of 60 fields was seldom reasonably representative of the sample from it came even when the bacterial content of the milk was rather high for such milk. The examination of 1000 fields sometimes gave misleading results. Indeed, in milks of low bacterial content 0.01 cc. cannot be depended upon as being truly representative. As milk improvement is effected the usefulness of this test as a precise quantitative measure decreases unless more fields are examined than seems possible in routine work. The writers are of the opinion that in the reporting of research a statement of the Breed count should always be accompanied by a citation of the number of examined fields unless the bacterial content of the milk is high. These results are in general accord with those reported by others for milk of low bacterial content (3, 5).

CONCLUSIONS

1. The high/low ratio of replicate 60 field direct microscopic leucocyte counts from Breed smears may be expected to be not greater than 3/1. This variation is interpreted as indicating reasonable accuracy in this method of counting leucocytes in milk.

2. The high/low ratio of the 60, 1000 and, in a few cases, 2000 field

direct microscopic counts of bacteria from the same sample was greater than 4/1 in 16 (72.7 per cent) and greater than 8/1 in 12 (54.5 per cent) of 22 milks of low bacterial content. This variation is interpreted as indicating that the 60 field direct microscopic count is not reasonably accurate as a precise estimate of the number of bacteria in this class of milk.

3. The high/low ratio of replicate 6000 field direct microscopic counts were 3.29/1 and 2.36/1 for two milks of low bacterial content. The interpretation is made that 0.01 cc. may not constitute a representative sample for the precise estimation of bacterial numbers in this class of milk.

4. The number of fields on which the direct microscopic count of bacterial numbers in milk is based should always be reported unless the milk is high in bacterial content.

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A COMPARISON OF THE STANDARD WITH THE MODIFIED METHYLENE BLUE REDUCTION TECHNIC

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INTRODUCTION

Since the first use of dye reduction as an indicator of bacterial action in milk (2, 5) general milk supplies on this continent have undergone remarkable improvement. Many of the earlier studies of the methylene blue reduction test were largely concerned with a class of milk which is today rapidly disappearing from the market.

With the gradual elimination of the poorer classes of milk from the fluid milk market the inaccuracies of the methylene blue reduction test evoke greater interest than heretofore. Attention has recently been focused on this subject by the acceptance of the recommendation of Wilson (12) that a modified methylene blue reduction technic be adopted as standard in England.

One of the outstanding advantages of the methylene blue reduction test is that, while the interpretation of results should be made only with an adequate knowledge of the available information regarding the test, the technic of operation may demand a less exact training than either the agar plate or microscopic counts. For this reason the methylene blue reduction test has been useful in many situations where otherwise bacteriological control of the milk supply would be difficult or impossible.

The modification proposed by Wilson complicates the operation of the test to the extent that strictly uniform technic would be improbable in the hands of some who are at present satisfactorily performing the test. Therefore, any extensive complication of the technic should be adopted as standard on this continent only on the presentation of proof that the modification compensates with greater accuracy.

HISTORICAL

In 1913 Skar (6), while studying the reduction of methylene blue by milk leucocytes, observed irregular disappearance of the dye from some samples of milk. He introduced a shaking technic which resulted in decreased reduction times of unreported magnitude. He concluded that bacteria and leucocytes are carried into the cream layer by the rising butterfat or collect by the force of gravity, or otherwise, at the bottom of the tube. The cells so situated would have, therefore, little or no effect upon the reduction time in the main body of the milk.

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Thornton and Hastings (9, 10) observed serious variations in the reduction times of replicate tubes of many milks, these variations tending to increase with the reduction times. The variation in dye reduction in different portions of the same tube as well as the variations in reduction times in replicate tubes disappeared almost completely and reduction times were shortened, particularly in the case of long-time reducing milks, when the tubes were shaken during incubation. They obtained the same results whenever the butterfat was prevented from rising whether by agitation, lack of space, rennet or agar coagulation or by homogenization. Violent, prolonged or frequent shaking gave approximately the same results as ordinary shaking. They concluded that in the standard technic butterfat rising in the unagitated milk sweeps bacteria into the cream layer, a larger proportion being so swept out of good than of poor milk. This, the authors believe, is a cause of variation and inaccuracy in the reduction test and the interpretation was made that this test should not be considered reasonably accurate after the 5½-hour period.

Johns (4) introduced a "modified" methylene blue reduction test, the modification being:

(a) Preliminary incubation at 55° F. (12.8° C.) for 18 hours, and
(b) Mixing contents of tubes not decolorized in 6 hours when subsequently incubated at blood heat.

The author commented that "the chief advantages of the modified test are: (1) greater convenience to the analyst, (2) improved accuracy on high grade milks and (3) closer correlation with keeping quality."

In 1934 Thornton *et al.* (11) reported that "The standard methylene blue reduction test was supplemented in almost all cases by a modified test. The modification consisted of shaking the tubes every half-hour during incubation in the constant-temperature water-bath which was maintained at 37° C. $\pm$ ½°. This technique has the effect of shortening the reduction times of the majority of milks of the class under discussion in this paper and, we believe, gives more nearly accurate results than the standard technique."

Wilson (12) recommended the adoption in England of a modified methylene blue reduction test, the modifications being inversion of the tubes once every half-hour during incubation, which is conducted in the dark. He agrees that the sweeping action of the rising butterfat is a cause of variation in the test and believes that the butterfat itself, "or some substance adherent to it," plays a part in the mechanism of reduction.

DEFINITION OF TERMS

In the present paper the standard methylene blue reduction test of the A.P.H.A. (1) will be referred to as the methylene blue reduction test while the modified methylene blue reduction test will mean one inversion of the

tubes of milk each half-hour during incubation with no consideration of the effect of light on reduction. Reduction times are reported in hours and minutes, 8:30 meaning 8 hours and 30 minutes.

THE SOURCES OF INACCURACY IN THE REDUCTION TEST

There are probably a number of sources of inaccuracy in the methylene blue reduction test, some perhaps still unrecognized. Of the recognized sources at least three appear to be important.

The first is dependent on the well-known fact that some living bacteria which occur in milk may be unable to grow in this medium at 37° C. What definite relation this bears to the accuracy of the methylene blue reduction test is not known.

The second source of inaccuracy depends on the varying influence of different species of bacteria which grow in milk at 37° C. on oxidation-reduction potential drifts. This is assumed to vary with the oxygen consumption rates of the bacteria and the extent of inaccuracy thus introduced into the test is not known.

The third important source of inaccuracy is the sweeping of bacteria out of the milk by the rising butterfat. An essential departure from the standard technic demanded by the three modifications mentioned above is in the nature of an effort to eliminate or control this factor.

Thornton and Hastings believed the methylene blue reduction test not to be reasonably accurate after the 5½ hour period, an opinion with which Wilson seems to concur. Johns on the other hand believes the test to be reasonably accurate up to 10 hours, basing his estimate of accuracy on variability in replicate tubes. Thornton and Hastings used decreased reduction times due to shaking as well as variability in replicate tubes as their criteria of accuracy. Thornton *et al.* (11) present evidence that, despite its inaccuracy when used on better class milks, the methylene blue reduction test is still more accurate for many of these milks than is the plate count. If this is a sound conclusion, then it is probable that the reduction test will continue to be used for good milks until a more accurate test is available. Nevertheless, it is questionable if intelligent interpretation can at present be made of standard reduction time differences in milks reducing beyond 10 hours.

VARIABILITY IN REPLICATE SAMPLES

Thornton and Hastings (10) report rather startling variations of reduction times in replicate tubes for a few samples of milk but fail to indicate the extent of such variations for 95 other milks. These variations recalculated from their data are here presented in Table 1 and are not essentially different from the variations reported by Johns (4).

The average generation time during the reduction test of commercial

TABLE 1

Relation between reduction time and variability
(Recalculated from data of Thornton and Hastings on 95 samples.)

CLASS INTERVAL IN HOURS	NUMBER OF SAMPLES	NUMBER OF SAMPLES SHOWING VARIATION IN DUPLICATE TUBES	MAXIMUM VARIATION	AVERAGE VARIATION
0– 1	11	0	0: 00	0: 00
1– 2	11	2	0: 35	0: 05
2– 3	7	0	0: 00	0: 00
3– 4	11	1	0: 25	0: 03
4– 5	10	1	0: 50	0: 05
5– 6	9	0	0: 00	0: 00
6– 7	8	1	0: 15	0: 02
7– 8	10	3	0: 40	0: 06
8– 9	8	4	0: 45	0: 13
9–10	5	5	0: 45	0: 30
10–12	5	5	0: 30	1: 01

milks is not known. The average generation times in the case of 25 aseptically-drawn milks were found in this laboratory to vary from 29 to 74 minutes, averaging 54 minutes. If we assume a generation time of 1 hour in the reduction test then a variation of 1 hour represents 100 per cent variation in the bacterial numbers of the original milk, irrespective of the reduction time. It is seen that not only does the inaccuracy of the reduction test increase with increasing reduction times but the inaccuracy due to this factor alone is a serious one.

Ellenberger *et al.* (3) and Wilson (12) concluded that the methylene blue reduction test is many times less variable than the plate count but failed to take cognizance of the fact that the variability displayed by the one method cannot be directly compared with that exhibited by the other because of logarithmic relations in the methylene blue test and linear relations in the plate count. Wilson's statement that the modified reduction test is 20 times less variable than the plate count becomes misleading in view of the method by which this figure was obtained. A coefficient of variability of 1.12 per cent of the mean reduction time, 235 minutes, is a standard deviation of 2.63 minutes. If a variation of 60 minutes in the reduction time represents 100 per cent variation in the bacterial content of the original milk and if the bacterial content and the plate count are assumed to be identical, then a variation of 2.63 minutes in the reduction test represents approximately 4.4 per cent variation in the plate count. With these assumptions the comparable figures are 4.4 per cent and 21.45 per cent which Wilson reported as the coefficient of variability of the plate count. In these terms the modified reduction test is approximately 5 times less variable than the plate count.

When calculated in a similar manner the methylene blue reduction test coefficient of variability of 4.47 per cent as reported by Ellenberger *et al.* (3, Series I, Table 5) is the equivalent of 58 per cent variation in the plate

count which is almost exactly double the coefficient of variability (29.02 per cent) of the plate counts of the same 79 samples of milk. Their statement that "the methylene blue reduction test shows much less (one-seventh as much) variability between check or duplicate tests than does the agar plate method" should be accepted with considerable reservation.

REDUCTION TIME DIFFERENCES DUE TO SHAKING

Table 2 presents average reduction time differences due to shaking for 335 market milks. It is seen that the modified technic results on the average

TABLE 2

Relation between standard and modified reduction times of 335 market milks

CLASS INTERVAL IN HOURS	NUMBER OF SAMPLES	AVERAGE STANDARD REDUCTION TIME	AVERAGE MODIFIED REDUCTION TIME	DIFFERENCE DUE TO SHAKING
0– 1	11	0: 30	0: 39	+ 0: 09
1– 2	17	1: 20	1: 17	– 0: 03
2– 3	10	2: 20	2: 08	– 0: 12
3– 4	15	3: 25	2: 47	– 0: 38
4– 5	24	4: 23	3: 29	– 0: 54
5– 6	37	5: 24	4: 11	– 1: 13
6– 7	29	6: 23	5: 07	– 1: 16
7– 8	35	7: 18	5: 50	– 1: 28
8– 9	28	8: 24	6: 11	– 2: 13
9–10	18	9: 25	6: 52	– 2: 33
10–11	29	10: 17	7: 13	– 3: 04
11–12	28	11: 26	6: 50	– 4: 36
12–13	31	12: 27	7: 38	– 4: 49
13–14	13	13: 17	7: 49	– 5: 28
14–15	9	14: 10	8: 21	– 5: 49
15–16	8	15: 30	9: 39	– 5: 51
16–17	1	16: 00	9: 00	– 7: 00
17–18	1	17: 00	7: 00	– 10: 00
23–24	1	23: 00	8: 00	– 15: 00

in little difference up to 2-3 hours and may even cause an increased reduction time in some of these milks due to the incorporation of oxygen just prior to reduction of the dye. In the medium and better class milks there is a material decrease in average reduction time due to the inversion of the tubes. These results check very closely with those of Johns and Wilson. If the modified test is substituted for the standard test it appears sound to use the following equivalents:

Standard reduction time	*Modified reduction time*
8: 00	6: 00
5: 30	4: 00
2: 00	2: 00

A total of 6 better class market milks in some hundreds of samples have been observed by the writer to have longer modified than standard reduction times (Table 3). The difference in the case of milk 3 is probably due to

shaking when the end-point was almost reached. At least 3 of the samples were found to contain mastitis milk. No information was available for

TABLE 3

Increased reduction times due to shaking 6 market milks reacting abnormally

MILK NUMBER	STANDARD REDUCTION TIME	MODIFIED REDUCTION TIME	INCREASE DUE TO SHAKING
1	4: 30	8: 00	3: 30
2	5: 15	7: 30	2: 15
3	5: 20	5: 45	0: 25
4	6: 00	8: 00	2: 00
5	6: 00	8: 30	2: 30
6	6: 30	8: 00	1: 30

the 2 remaining milks to permit of interpretation.

As milk supplies on this continent improve they are gradually approaching mixed aseptically-drawn milks. The effect of shaking such milks during the reduction test is, therefore, of importance. Table 4 contains data on

TABLE 4

The effect of shaking 95 aseptically-drawn udder milks

CLASS INTERVAL IN HOURS	NUMBER OF SAMPLES	AVERAGE STANDARD REDUCTION TIME	AVERAGE MODIFIED REDUCTION TIME	AVERAGE DIFFERENCE
0– 1	4	0: 40	0: 40	0: 00
1– 2	2	1: 45	1: 50	+0: 10
2– 3	1	2: 10	2: 15	+0: 05
3– 4	4	3: 22	2: 53	−0: 29
5– 6	2	5: 00	6: 00	+1: 00
6– 7	1	6: 15	14: 45	+8: 30
7– 8	1	7: 00	6: 30	−0: 30
8– 9	5	8: 27	6: 27	−2: 00
9–10	5	9: 24	7: 22	−2: 02
10–11	5	10: 14	8: 45	−1: 29
11–12	4	11: 25	9: 30	−1: 55
12–13	3	12: 35	10: 08	−2: 27
over 13	58	over 13: 00	10: 46	over −3: 00

95 samples drawn aseptically from the udder, the sample being a composite from each milking quarter of a cow. The milks reported in Table 5 were aseptically drawn from each milking quarter of 27 cows on each of three mornings within one week, the milk from each quarter being tested separately. Strynadka, from whose work these data were taken (7), showed that when the shaking technic results in a considerably prolonged reduction time an abnormal condition of the udder is indicated. The effect of such abnormal milk on the methylene blue reduction test as well as the modified test is lessened as it is mixed with normal milk. It is believed, therefore, that the conclusions reached in this communication regarding the use of the modified test for present market milks will continue to be sound as the milk supply improves.

TABLE 5

The effect of shaking 313 aseptically-drawn milks from the individual quarters of 27 cows

CLASS INTERVALS IN HOURS	NUMBER OF SAMPLES	AVERAGE STANDARD REDUCTION TIME	AVERAGE MODIFIED REDUCTION TIME	AVERAGE DIFFERENCE
0– 1	1	0: 30	0: 30	0: 00
1– 2	3	1: 10	1: 55	+ 0: 45
2– 3	2	2: 00	1: 30	– 0: 30
3– 4	4	3: 15	3: 41	+ 0: 26
4– 5	2	4: 38	3: 15	– 1: 23
5– 6	4	5: 23	5: 56	+ 0: 33
6– 7	6	6: 25	9: 28	+ 3: 03
7– 8	3	7: 30	4: 00	– 3: 30
8– 9	5	8: 15	7: 18	– 0: 57
9–10	2	9: 15	7: 30	– 1: 45
10–11	15	10: 18	8: 16	– 2: 02
11–12	18	11: 37	8: 56	– 2: 41
12–13	21	12: 26	8: 56	– 3: 30
13–14	23	13: 15	8: 53	– 4: 22
14–15	16	14: 29	9: 56	– 4: 33
15–16	22	15: 23	10: 12	– 5: 11
16–17	33	16: 22	9: 50	– 6: 32
17–18	18	17: 23	9: 34	– 7: 46
18–19	15	18: 03	10: 20	– 7: 43
19–20	9	19: 25	11: 32	– 7: 55
20–21	22	20: 16	10: 03	– 10: 13
21–22	13	21: 23	10: 38	– 10: 45
22–23	9	22: 07	11: 13	– 10: 54
23–24	7	23: 21	11: 08	– 12: 13
24–25	20	24: 06	11: 54	– 12: 12
over 25	20	over 25: 00	11: 47	– 13: 13

FACTORS INTRODUCED INTO THE TEST BY THE SHAKING TECHNIC

There appear to be at least three factors introduced into the test by shaking the tubes, viz.:

1. The bacteria are kept more evenly distributed.
2. The butterfat is kept more evenly distributed.
3. More oxygen becomes dissolved in the milk.

The bulk of the evidence supports the theory of Thornton and Hastings that the decrease in reduction time due to shaking is largely attributable to a more even distribution of bacteria.

It has never been proved that the butterfat itself is in no way directly concerned with the reduction of methylene blue in milk. Wilson states that "The effect of the fat is still undetermined, and until it can be obtained in pure condition, freed from all dissolved and adsorbed substances, and emulsified in a legitimate manner, opinion must be reserved on its real action. It appears, however, to have at least three effects. In the first place it probably acts in a purely physical capacity, affording a large surface on which enzyme reactions can occur. In this connection it may be noted that both xanthine oxidase, and the other enzymes of whose existence we have some evidence, are both linked to the fat. Secondly, it has a visual effect, de-

creasing the depth of colour yielded by a given concentration of methylene blue, and consequently shortening the time to apparent complete reduction of the dye. Thirdly, by adsorbing methylene white, it disturbs the ratio of oxidant to reductant, and so allows complete reduction of methylene blue to occur at a higher Eh than would have been registered in the absence of fat."

If this opinion is right it is difficult to explain the results of Thornton and Hastings with homogenized milk and would seem to imply a condemnation of the modified reduction test as well as the application of the reduction test to cream. Neither group of workers was able to correlate reduction times and fat contents of market milks. Thornton (8) presented evidence that the sweeping effect of the rising butterfat is related to the creaming properties of the milk, one milk reported by him reducing in 2:30 by the standard technic and in 6:25 when manipulated so as to enhance creaming.

The incorporation of extra oxygen into the milk through shaking has at least a twofold effect as pointed out by Thornton and Hastings. It has a delaying effect inasmuch as the bacteria are required to consume more oxygen. It may also stimulate or retard bacterial growth. Wilson believes the stimulating effect of importance but fails to report by what technic he was able to measure it. His work with 10 and 20 ml. quantities of milk permit of two interpretations, viz.: oxygen effect and enhanced creaming effect. It seems probable that the latter was the more important.

It is highly improbable that the stimulating effect of the added oxygen is a major factor in the modified test because half of the total oxygen consumed during the test is consumed during the last generation period before reduction, assuming constant bacterial growth. In a long-time reducing milk the oxygen pressure is, therefore, very slightly decreased for the larger part of the test. The fact that the modified technic does not often result in shortened reduction times of the poorer milks does not point to stimulation of bacterial growth during the period of rapid fall of potential.

If the incorporation of extra oxygen is effected immediately prior to reduction of the dye, the reduction time is thereby lengthened. Thus care should be exercised in the final inversion of the tube.

FREQUENT VERSUS INFREQUENT SHAKING

Thornton and Hastings found no difference in reduction times when the tubes were shaken gently (2 to 3 times) or violently (25 times) every half-hour, while quarter- and half-hour shakings gave different results only in the case of short-time reducing milks.

Johns observed differences in the case of some milks between shaking every hour and every 3 hours, while shaking every 6 hours gave materially different results.

Wilson reported a mean difference of 8 minutes for 19 samples of Grade A milk when shaken once and 4 times every half-hour. He concludes that "The extra inversion of the second set had thus delayed reduction for about 8 minutes." Since it is probable that this difference is not outside the experimental error even of reading the end-point of reduction for this class of milk, the author's statement hardly seems justifiable. His further results (12, Table CXXVIII) should be interpreted with care for he worked with a small number of samples, the groups were not composed entirely of replicates and the differences between group reduction times were not, in some cases, outside the standard deviations of the differences. By the usual criterion of significance for a small number of samples (Fisher's t) a difference of about 24 minutes would be necessary to be significant.

Data on a few samples of milk subjected to varying shaking technics are presented in Table 6.

TABLE 6

The effect of varying frequency and violence of shaking

MILK NO.	NO. OF TUBES	STANDARD		INVERTED ONCE AT 4–6–8 HOURS		INVERTED ONCE EACH HALF HOUR	
		Average red. time	Maximum variation	Average red. time	Maximum variation	Average red. time	Maximum variation
1	5	12: 30	1: 00	8: 15	0: 00	7: 00	0: 00
2	5	8: 30	0: 00	7: 00	0: 00	6: 30	0: 00
3	5	13: 15	0: 30	9: 15	0: 00	8: 00	0: 00
4	5	6: 45	0: 00	5: 00	0: 00	4: 30	0: 00
				Violently shaken at 4–6–8 hours			
5	5	11: 00	1: 00	6: 45	0: 00	6: 00	0: 00
6	5	8: 15	1: 00	5: 15	0: 00	4: 30	0: 00
7	5	12: 00	1: 00	8: 45	0: 45	7: 15	0: 00
8	5	8: 30	0: 45	6: 30	0: 00	5: 45	0: 00
				Inverted once each hour			
9	5	8: 45	1: 15	5: 45	0: 00	5: 30	0: 00
10	5	9: 45	0: 15	6: 00	0: 00	6: 00	0: 00
11	5	7: 00	0: 00	6: 15	0: 00	6: 15	0: 00
12	5	7: 25	0: 15	6: 00	0: 00	6: 00	0: 00

The weight of the available evidence is to the effect that inversion of the tubes once per hour gives results differing from inversion once each half-hour, if at all, by an amount that is within the experimental error of reading the end-point. The shaking technic, if used, should be of sufficient force and frequency to prevent the formation of a cream layer or ring difficult to disperse.

THE ACCURACY OF THE MODIFIED TEST

Attempts to determine the accuracy of the methylene blue reduction test have been made by a large number of investigators using various criteria. The most frequently used criteria have been the agar plate count, the keeping

quality of the milk and the variability of the reduction test. Such methods of comparison have not as yet resulted in a proved accuracy for any of the three tests. It is probable that the methylene blue reduction test is at least as accurate as the plate count and more accurate than the keeping quality test as a measure of the numbers of bacteria in raw milk. The determination of the accuracy of one method of measurement by comparison with another method of unknown accuracy appears to be futile.

Some workers are willing to accept the variability of the reduction test or plate count as a complete measure of accuracy. The present writer is of the opinion that variability is a very inadequate measure of the accuracy of the plate count and probably of the reduction test.

Thornton and Hastings (10), Johns (4), Thornton (8), and Wilson (12) have all expressed the belief that the shaking technic results in greater accuracy in the methylene blue reduction test but none has yet substantiated his opinion with acceptable proof or successfully measured the extent of greater accuracy.

The coefficient of correlation between the methylene blue reduction times and the modified reduction times of 332 of the 335 market milks reported in table 4 is 0.94 ± 0.004. This very high correlation means that on the average there can be little difference in the accuracy of the two tests, although the variability displayed by the standard test may lead to inaccuracy in the case of an individual sample.

CONCLUSIONS

This review of the literature and presentation of new data seem to justify the following conclusions:

1. The accuracy of the methylene blue reduction test still awaits determination.

2. Replicate samples of good milks frequently exhibit serious variations of standard reduction times.

3. The variations tend to increase in frequency and magnitude as the reduction time increases.

4. These variations practically disappear if the tubes of milk are shaken during incubation and the reduction times of such milks are usually shortened.

5. The coefficient of correlation between the standard and modified reduction times is so high that there is little, if any, difference in the average accuracies of the two tests despite the variability in individual samples in the standard test.

6. The present evidence does not warrant the replacement as standard of the methylene blue reduction test by the modified methylene blue reduction test, since the possible greater accuracy of the latter is offset by greater complexity of technic.

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THE RESAZURIN TEST—ITS USE AND PRACTICABILITY AS APPLIED TO THE QUALITY CONTROL OF RAW MILK

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An earlier report[1] by Ramsdell, Johnson, and Evans discussed the use of Resazurin as a chemical indicator for determining the sanitary quality of milk. On the basis of the above investigation, it was concluded that the use of Resazurin gave more information as to the quality of milk than any other chemical indicator now in use. Further work on this dye was conducted by C. K. Johns.[2] Coincident with this latter research, investigations on Resazurin were initiated in these laboratories. It is the purpose of this paper to present data which indicate, first, that the Resazurin test is of added value as an indicator of quality over the Methylene Blue test as well as having the advantage of consuming much less time than the latter test, and second, that the Resazurin test is a valuable adjunct to microscopic diagnosis in routine quality control of milk.

EXPERIMENTAL

Of primary interest was a comparison of the sensitivity of Resazurin to that of Methylene Blue, the standard plate method, and the microscopic Breed smear. Accordingly, the producer samples were obtained at the weigh can and all of the above-mentioned tests were run on each milk. The Resazurin test was run according to the following procedure:

1. One tenth of one cc. of 0.05 per cent Resazurin dye solution (Eastman) was measured into a sterile test tube.
2. Milk samples were obtained directly from the weigh can, using a 10 cc. narrow bore-type dipper.
3. Samples were incubated for one hour at 98° F., in a covered water bath.
4. The samples were read and recorded as quickly as possible after the incubation period.

A total of 305 samples were collected for this test over a period of five weeks. Although the determinations were made in unison, they are presented separately for purposes of clarity.

Of the 220 samples remaining blue at the end of one hour of incubation with Resazurin, 201, or 91 per cent, were incapable of reducing the Methy-

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[1] Ramsdell, G. A., Johnson, Jr., W. T., and Evans, F. R. Investigation of Resazurin as an indicator of the sanitary condition of milk. JOUR. DAIRY SCI., 18: 705–717. 1935.

[2] Johns, C. K. Paper presented at meeting of Vermont Dairy Plant Operators and Managers' Association, November 19, 1936.

TABLE 1

Comparison of color of Resazurin dye at the end of one hour with reduction time of Methylene Blue

NO. OF SAMPLES	COLOR OF RESAZURIN AFTER 1 HR.	REDUCTION TIME OF METHYLENE BLUE			
		7 hrs. or longer	6 hrs.	5 hrs.	Less than 5 hrs.
220	Blue	201	11	4	4
30	Purple Pink	21	3	1	5
32	Sl. Pink	26	3	1	2
14	Pink	4	2	1	7
7	Vivid Pink	4			3
2	White				2

lene Blue dye in seven hours or more. Purple pink and slight pink color shades, denoting some reduction with the Resazurin dye, did not, in many cases, affect the reduction time of Methylene Blue. This may be accounted for from the fact that staphylococci have a particular aptitude for reducing the Resazurin as compared to other types of bacteria commonly found in milk. Leucocytes encountered in colostral and mastitis-infected milk also cause rapid decolorization. The latter statement was particularly emphasized in the seven samples which were vivid pink on the Resazurin test at the end of one hour. Four of these samples which were unable to decolorize Methylene Blue in seven or more hours had a high leucocyte content. This bespeaks the sensitivity of Resazurin to physiologically abnormal and pathological milks as previously demonstrated.[1]

TABLE 2

Comparison of color of Resazurin dye at the end of one hour with standard plate count

NO. OF SAMPLES	COLOR OF RESAZURIN AFTER 1 HR.	STANDARD PLATE COUNT				
		Less than 25,000	25,000 to 50,000	50,000 to 100,000	100,000 to 200,000	Over 200,000
220	Blue	160	32	14	9	5
30	Purple Pink	7	12	3	3	5
32	Sl. Pink	17	10		2	2
14	Pink	8		2	2	2
7	Vivid Pink	4	1			2
2	White	1				1

The standard plate counts on these milks were less than 25,000 bacteria per cubic centimeter (Table 2). With five exceptions, milks incubated with Resazurin which remained unchanged in color at the end of the hour had a standard plate count of less than 200,000 per cc. The similarity in correlation of the purple pink and slight pink shades indicates the desirability of classifying these two colors together. One of the two samples completely reduced on Resazurin to white had a standard plate count of less than 25,000; however, the microscopic count was over 200,000, actually uncountable, as may be seen from Table 4. Again, the effect of staphylococci and high leucocyte milk on the reducing time of the dye was encountered, making

further correlations of this new test with previously known standards rather difficult. As may be seen from Tables 2 and 3 the Resazurin test is comparable to the Methylene Blue test in regard to the standard plate count.

TABLE 3

Comparison of reduction time of Methylene Blue with bacterial plate count

NO. OF SAMPLES	REDUCTION TIME OF METH. BLUE	STANDARD PLATE COUNT				
		Under 25,000	25,000 to 50,000	50,000 to 100,000	100,000 to 200,000	Over 200,000
253	7 hrs.	174	48	13	12	6
19	6 hrs.	6	5	3	3	2
7	5 hrs.	2	1	2		2
23	Under 5 hrs.	4	5	2	3	8

From these results it may be said that data as to the bacterial quality of milk obtained from one hour of incubation with Resazurin are fully as reliable as seven hours' incubation with Methylene Blue. Moreover, Resazurin is more sensitive to physiologically abnormal and pathological milks than is Methylene Blue. The correlation between Resazurin sensitivity and microscopic Breed count is better than that obtained through comparison with the standard plate method, as might be expected, due to the ability of the microscope to diagnose leucocytes, staphylococci, etc.

TABLE 4

Correlation between colors developed after one hour of incubation with Resazurin and microscopic Breed count

NO. OF SAMPLES	COLOR OF RESAZURIN AFTER 1 HR.	MICROSCOPIC BREED COUNT				
		Under 25,000	25,000 to 50,000	50,000 to 100,000	100,000 to 200,000	Over 200,000
166	Blue	148	7	9	2	
25	Purple Pink		20	4	1	
32	Sl. Pink		22	3	5	1
11	Pink		7	2	1	1
7	Vivid Pink	4	1		1	1
2	White*					2

* High leucocytes.

It, therefore, cannot be stated that an accurate or semi-accurate bacteria count can be obtained from Resazurin because it is very sensitive to milk that is abnormal from causes other than bacteria count. Yet, the very sensitivity of Resazurin to these other quality defects in milk should be characterized as an asset.

If reliance were placed on the classification of milk according to the various color shades of Resazurin formed at the end of the hour of incubation considerable discrepancy between laboratories could be expected unless color standards were used. Microscopic diagnosis of all samples showing partial or complete reduction therefore seemed desirable. Moreover, in many plants

microscopic examination of individual producers' milk constitutes a great portion of the time spent in laboratory control. A preliminary test which would be capable of segregating those milks which are satisfactory from those which are not, prior to microscopic diagnosis, would place emphasis on poor milks and also save a considerable amount of time.

THE RESAZURIN TEST AS A PRELIMINARY TEST FOR MICROSCOPIC DIAGNOSIS

All producers' samples were incubated with Resazurin for one hour. At the termination of this period the samples were examined and Breed smears were made directly from those tubes in which the dye was partially or completely reduced. This method was adopted inasmuch as it obviates the necessity of double samples and since we were more interested in determining the types of bacteria rather than the total number of bacteria. The slight growth obtained during the incubation period was of value particularly in those cases where *Streptococcus agalactiae* was the cause of reduction. The practical application of this procedure is demonstrated in Table 5, a duplication of a test sheet from one of our country stations. This table represents the number of samples examined out of 100 producer samples taken.

TABLE 5

Microscopic examination of milk found positive to Resazurin test

PRODUCER NUMBER	COLOR OF RESAZURIN AFTER 1 HR. INCUBATION	MICROSCOPIC DIAGNOSIS	REMARKS
5	Slight Pink	120,000 bact. per cc. 900,000 " " "	Utensil bacteria
8	Vivid Pink		Utensil and lactic bacteria types
14	White	Uncountable	Spore formers and lactics. Check immediately
20	Purple Pink	25,000 bact. per cc. 1,500,000 leucocytes "	
21	Purple Pink	50,000 bact. per cc.	Utensil and lactics
38	Pink	25,000 " " " 4,000,000 leuco. " "	Mononuclear types. Hold out milk from fresh cows
59	Vivid Pink	Mastitis	Reject milk until cleared up. Check herd
62	Purple Pink	250,000 bact. per cc.	Utensil and lactics
70	Purple Pink	O. K.	
76	White	Uncountable	External contamination combined with dirty utensils and poor cooling. Check immediately
80	Purple Pink	30,000 bact. per cc. 2,000,000 leuco. " "	Staphylococci. Check milking machine in particular
87	Purple Pink	O. K.	
90	Pink	O. K.	
96	Vivid Pink	600,000 bact. per cc. 3,000,000 leuco. " "	Lactics

In most cases where there was a color change, as in the above table, poor quality was verified by the microscope. Occasionally samples will change

to a purplish pink and may even turn pink, yet under the microscope no abnormality is observed. The cause of this has not as yet been entirely determined. However, previous investigations[1] state that the Resazurin dye is reduced more rapidly by milk with a high catalase and chloride content and these factors may be responsible.

As may be observed from Table 5, the time saved through the use of the Resazurin-microscope combined procedure was equivalent to the time necessary to smear, diagnose and wash up the slides for 86 microscopic samples.

An approximation of the amount of time saved in our other country laboratories is given in Table 6.

TABLE 6

STATION	NO. OF SAMPLES TAKEN IN 1 WK.	MICROSCOPIC ANALYSES MADE 1 WEEK	ESTIMATED TIME SAVED 1 WEEK
A	475	75	13 hrs.
B	600	100	16 hrs.
C	260	40	6 hrs.
D	400	60	11 hrs.
E	365	55	10½ hrs.
F	260	40	6 hrs.
G	240	35	5½ hrs.
H	120	20	2 hrs.

Thus, the introduction and use of the procedure in our country laboratories has given us quicker segregation of poor quality milk, placed more emphasis on poor quality milk, allowed more time for follow-up and, because of this, increased the frequency with which the tests can be run. For these reasons the combined Resazurin-microscopic diagnosis seems to be of singular aid in a general quality improvement program.

These preliminary thoughts on the use and practicability of Resazurin are presented that they might stimulate further discussions and research on a test which warrants further investigation.

CONCLUSIONS

1. Information as to the sanitary quality of milk can be obtained in one hour through the use of the Resazurin test which is comparable to that obtained in seven hours using the Methylene Blue test.

2. The Resazurin test is superior to the Methylene Blue test in that it is extremely sensitive to physiologically abnormal and pathological milks.

3. The Resazurin test is a valuable adjunct to microscopic diagnosis in eliminating the time normally spent in diagnosis of good milks, thereby allowing more time for detection of the source of the trouble with poor milks.

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THE DETECTION OF SHEDDERS OF THE STREPTOCOCCUS OF MASTITIS IN COMPOSITE CONTROL MILK SAMPLES[1]

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In the examination of milk samples for the purpose of control, high cell counts and streptococci indicate that routine dairy inspection, including physical examination of animals and chemical tests (1) on the milk, does not detect all shedders of the streptococcus of mastitis. It has been shown by the most recent investigators (Bryan (2), Hucker and Hansen (3), Minett (4), and Plastridge, Anderson, Brigham and Spaulding (5)) that about 90 per cent of the strains causing infectious bovine mastitis belong to a well-defined group to which the specific name *Streptococcus agalactiae* has been given.

While the characteristics of *Streptococcus agalactiae* (6) are well known, it is sometimes difficult for the laboratory, using as samples milk from individual cows, to do the work necessary to its identification. The purpose of this paper is to describe a routine procedure which can be carried out in a public health laboratory, using composite producer-control samples, and to demonstrate that the quality of the milk supply can be improved by the application of this procedure and consequent elimination of infected individuals in the herd.

Direct microscopic tests have been described in which the finding of long-chained streptococci in aseptically-drawn individual samples were thought to indicate the existence of an infection (1, 2). Our findings are not in agreement with those findings since saprophytic streptococci have been isolated repeatedly from aseptically-drawn individual samples and *Streptococcus agalactiae* has been found in samples in which no streptococci were seen in smears from either unincubated or incubated samples.

A satisfactory method for the detection of animals infected with mastitis should include, not only the demonstration of the presence of streptococci in the udder secretions, but also the identification of the type or types found. Although we realize the importance of high cell counts in individual cow samples, we do not believe they have the same significance when made from composite herd samples. However, in many instances we recorded very high cell counts from the composite samples and took this to indicate the possible existence of mastitis infection in the herd. Since both the finding of long-chained streptococci and high cell counts in smears made from com-

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posite herd samples indicate infection of herd members, further examination of such samples seemed important.

METHODS

All composite control samples revealing long-chained streptococci, or having cell counts of 500,000 or more were retained under refrigeration for the isolation and identification of streptococci. The identification of these streptococci indicated which herds contained infected animals and led to the examination of samples from the individual cows in suspected herds.

The water used for rinsing the Breed pipette between the control samples contained about 50 p.p.m. of chlorine which prevented contamination of one sample by a preceding one.

In most of the tests reported in this paper the samples containing streptococci or many cells were incubated from 4 to 8 hours at 37° C., before streaking on blood agar plates. It was found that many of the plates were so overgrown with gram-negative bacilli that isolation of streptococcus colonies was difficult. All composite producer samples were thereafter inoculated into 10 cc. of fresh sterile 1 per cent sodium carbonate solution, using 2 cc. of the milk sample instead of 1 cc., as suggested by Groesbeck (7) and incubated over-night at 37° C. The samples from individual cows, aseptically drawn, were incubated over-night at 37° C., in their original containers. Blood agar plates were streaked from these incubated samples and incubated at 37° C., for 24 hours. Stained microscopic preparations were made also from the incubated samples. These were examined in order to determine whether an increase in the number of streptococci occurred in the sample upon incubation, and to compare the morphology and colonial appearance of unknown and known strains. Typical streptococcus colonies producing any degree of hemolysis on the streaked blood agar plates were transferred to litmus milk and incubated at 37° C., over-night. Sometimes as many as four colonies were transferred to separate tubes of litmus milk from a single blood agar plate. Microscopic examination of stained smears from these litmus milk cultures was made to determine whether they were pure cultures of streptococci. Transfers were made from the pure cultures in litmus milk to sodium hippurate and esculin broths and incubated at 37° C., for 72 hours. Sodium hippurate and esculin broths of several different compositions were tried before uniform results were obtained as follows:

.1% of esculin in Douglas broth (8)[2]

1% of sodium hippurate in Douglas broth[2]

The original formula and method of Ayers and Rupp (9) was not productive of good growth with all strains of streptococci. Approximately 4 cc. of each medium was tubed for use. To the culture in sodium hippurate was added 1 cc. of a 2 per cent ferric chloride solution in 2 per cent HCl. A positive reaction, the splitting of sodium hippurate, was indicated by a warm-brown

[2] Good growth was obtained also with beef infusion broth.

precipitate. An uninoculated control tube and one inoculated with a known "hippurate-positive" culture accompanied each day's work on unknown samples. About 0.2 cc. of a 1 per cent aqueous solution of ferric citrate was added to the culture in esculin broth. The splitting of esculin, a positive reaction, was indicated by a blackening of the culture upon addition of the reagent. The original fluorescence of the esculin medium was not destroyed by the growth of *Streptococcus agalactiae*. Media containing trehalose and sorbitol were employed in the differentiation of human and animal strains of beta-hemolytic, low-acid producing, hippurate-negative strains of streptococci. Transfers were made to both trehalose and sorbitol media from the litmus milk that gave a slight acid reaction. The production of acid in the trehalose or sorbitol media indicated a positive reaction. The base for this media was made by adding to Douglis broth[3] 10 per cent beef serum and 1 per cent Andrade's indicator. To the base was added 0.5 per cent of trehalose or sorbitol, as desired. These media were tubed in 2 cc. quantities.

In order to determine whether or not routine control samples were to be retained for isolation and identification of the streptococci, it was not necessary in all cases to find long-chained streptococci in the original smear. Many of the samples from which *Streptococcus agalactiae* were isolated revealed only short chains in the unincubated direct smears. We have found that the size of the individual cells and their arrangement in the chain are of more importance than the length of the chain. Smears for microscopic examination made from litmus milk with the typical curdled reaction of *Streptococcus agalactiae* usually contained broken chains of two, three or four units in length. For this reason, smears from litmus milk were made as soon as growth was evident. Re-inoculations from the curdled litmus milk into broth has produced chains of fifteen or more units in length. The characteristics of the streptococci most often found in milk are given in Table 1. The most characteristic reaction occurs in litmus milk in which over 95 per cent of the strains produce the (ACR) reaction in from 15 to 48 hrs. of incubation. A few of the strains of *Streptococcus agalactiae* produce acid in litmus milk without curdling it. These variant strains of *Streptococcus agalactiae* differ from *Streptococcus agalactiae* in that they ferment sorbitol and do not curdle litmus milk. The large number of saprophytic strains of streptococci encountered in the routine examination of producer samples also give a very characteristic reaction in litmus milk. The reduction of the color of the dye almost to the surface of the media before curdling, results in a white curdled milk with a narrow red band at the top. Saprophytic strains usually produced much shorter chains than those of *Streptococcus agalactiae;* the units were larger and were not so closely arranged in the chains, many of them having a "diplo" arrangement.

An acid litmus milk reaction without curdling is produced by the actively

[3] Good growth was obtained also with beef infusion broth.

TABLE 1

Cultural characteristics of streptococci found in milk

ORGANISM	STREAKED BLOOD AGAR PLATE	LITMUS MILK	SODIUM HIPPURATE	ESCULIN	TREHALOSE	SORBITOL
Streptococcus agalactiae	alpha alpha prime gamma	ACR	+	−		−
Variant of *Streptococcus agalactiae*	alpha	A no C	+	−		+
Streptococcus uberis	alpha	A no C	+	+		+
Streptococcus lactis	gamma	ARC	−	+		
Streptococcus fecalis	gamma	ARC	−	+		
Streptococcus viridans	alpha	A no C	−	±		
Streptococcus hemolyticus (human origin)	beta	Slight A no C	−	−	+	−
Streptococcus hemolyticus (animal origin)	beta	A no C	−	−	−	+

ACR = Acid, slight reduction of color from bottom after curdling.
ARC = Acid, reduction of color nearly to top before curdling.
A no C = Acid, no curd.

hemolytic (beta) colonies and by the green producing (alpha) colonies. This "Acid-no-Curd" reaction is typical of the important group of streptococci which include the human pathogens. The alpha strains produce enough acid from lactose to impart a distinct red color to litmus milk in 24 hrs., while the beta strains rarely make a noticeable change in the color of the litmus milk before two or three days of incubation. All true *Streptococcus agalactiae* strains are negative in sorbitol, while some few variant strains are positive. These sorbitol reactions should not be confused with the sorbitol and trehalose tests of Edwards (10) for the differentiation of human and animal strains of actively beta hemolytic, hippurate-negative streptococci, in which the human (beta) strains ferment trehalose but not sorbitol and the animal strains ferment sorbitol but not trehalose. The splitting of the glucoside, esculin, is characteristic of the saprophytic and fecal strains of streptococci. However, some human strains of *Streptococcus viridans* attack this carbohydrate but they can be separated by their reaction in litmus milk ("Acid-no-Curd").

TABLE 2

Classification of the 441 strains of Streptococci found in the 630 routine control samples in which long chain streptococci or cell counts over 500,000 per cc. were observed

	Streptococcus agalactiae	Variant of *Streptococcus agalactiae*	*Streptococcus uberis*	*Streptococcus lactis* *Streptococcus fecalis*	*Streptococcus viridans* (human)	*Streptococcus hemolyticus* (human)	*Streptococcus hemolyticus* (animal)
Number	228	12	25	157	18	1.0	0
Per cent	52	3	6	34	5	0.2	0

Table 2 shows the number of each of the seven types of streptococci isolated in this study of 630 samples selected from approximately 6000 routine producer control samples. From the 630 samples, 441 strains of streptococci were isolated, 228 (52%) were *Streptococcus agalactiae,* 12 (3%) a variant of *Streptococcus agalactiae,* 25 (6%) *Streptococcus uberis,* 157 (34%) *Streptococcus lactis,* 18 (5%) *Streptococcus viridans* (human) and one strain of *Streptococcus hemolyticus* of human origin.

All but one of the 228 strains of *Streptococcus agalactiae* produced the (ACR) litmus milk reactions. This one strain differed from *Streptococcus agalactiae* in that it did not curdle litmus milk but was unlike the variant strains since sorbitol was not fermented.

One hundred and forty of the strains of *Streptococcus agalactiae* were obtained from samples giving high cell counts (over 500,000 per cc.) and showing streptococci in the direct microscopic smear of the unincubated samples; 60 were from samples showing streptococci and low cell counts (under 500,000 per cc.) and 28 were from samples with high cell counts only. Besides the 28 strains of *Streptococcus agalactiae* isolated from the high cell count samples, 5 samples yielded strains of *Streptococcus lactis,* 4, strains of *Streptococcus uberis* and 2, strains of *Streptococcus viridans* (human).

Although approximately 50 per cent of the *Streptococcus agalactiae* were from samples in which long chained streptococci predominated in both the unincubated and incubated microscopic smears, the finding of long chained streptococci was not always indicative of *Streptococcus agalactiae* as 35 per cent of the *Streptococcus lactis* and *Streptococcus fecalis* strains produced long chains in the microscopic milk smears. Over 40 per cent of the *Streptococcus agalactiae* were from samples containing short chains. These strains grew in short chains in pure culture. The morphology of the strains of streptococci from milk was not characteristic. This was evident when notice was taken of the changes produced in some stock cultures grown on

different types of media and incubated and stored at different temperatures. *Streptococcus agalactiae* were isolated from 28 samples in which no streptococci were found in the unincubated microscopic smear. Colonial appearance, exclusive of hemolysis, contributes little to the identity of milk streptococci. Likewise the type of hemolysis on streaked blood agar plates after 24 hrs. of incubation was no indication of the identity of different strains of *Streptococcus agalactiae* since—83 strains of the *Streptococcus agalactiae* were from alpha colonies.

96 strains of the *Streptococcus agalactiae* were from alpha prime colonies.

49 strains of the *Streptococcus agalactiae* were from non-hemolytic (gamma) colonies.

Our work did not agree with that of Frost (11) in that no actively hemolytic (beta) mastitis strains were found in this study. This may be due to differences in the technic of making the blood agar cultures.

The laboratory detection of mastitis infection which for the most part was not recognized by physical inspection of animals and the "Brom Thymol Blue Tests" indicates the value of the routine laboratory procedure. Tests on samples from individual cows were made when a composite test indicated infection in the herd, or when infection was suspected by the producer or inspector. Elimination of the infected animals or at least their segregation at the end of the milking line was recommended. In most herds by complying with these recommendations there was a noticeable decrease in the number of streptococci and cell count of the herd milk and apparently less spread of infection in the herd.

In this study we found 42 badly infected herds. So far, individual cow samples have been examined from eleven of these herds and 48 animals have been removed from the milking lines. In 2 of these herds where the animals were not removed, the disease spread throughout the entire herd within a year's time. After identification of *Streptococcus agalactiae* from the composite milk of one herd, individual cow samples were examined from the 22 animals in the herd. Eight were found which were shedding *Streptococcus agalactiae* but showed no physical symptoms of the disease. The animals were not removed from the herd and after approximately a year the producer was not permitted to ship milk for fluid consumption because of consistently high counts. In a second herd from which it was recommended that 6 animals be removed, failure to remove these animals resulted in the loss of the entire herd in less than a year's time.

During the summer months many producers were degraded because of high bacterial counts. In many of the smears prepared from these samples, streptococci were observed and isolated. Examination of samples from individual cows revealed the offending animals which were removed from the milking line with a subsequent regrading of the producers.

A producer of grade "A" raw milk was degraded because of high bacterial counts. Inspection of equipment and animals and supervision of his methods of production caused no lowering of the counts. Samples from individual cows were examined. Seven of the 25 cows were found shedding streptococci in numbers sufficient to cause the composite milk to be degraded. The removal of these animals from the milking line likewise caused a subsequent regrading of the producer.

CONCLUSIONS

A series of cultural tests has been used routinely in a public health laboratory by which the identity of streptococci occurring in raw composite control milk samples has been determined.

These tests consist of a preliminary incubation of the sample and subsequent streaking on blood agar, from which the hemolytic streptococcus colonies are transferred to litmus milk. Plantings are made from the pure cultures, having characteristic reactions in litmus milk, to sodium hippurate and esculin broths. The beta hemolytic strains are tested for their ability to utilize trehalose and sorbitol.

When used routinely, with control samples, these tests supply a rapid method of locating herds containing animals with mastitis infections.

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A COMPARISON OF STANDARD PLATE COUNTS AND METHYLENE BLUE REDUCTION TESTS MADE ON RAW MILK WITH SPECIAL REFERENCE TO GEOMETRIC MEANS[1]

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It has been demonstrated (1) that the logarithmic or geometric mean is fairer and truer than the arithmetic mean in averaging plate counts made on milk samples. The geometric mean gives proper credit to the fact that bacteria multiply in a geometric progression. Also, the occasional extremely high or low count is more fairly handled when the geometric mean is obtained.

In an effort to determine the geometric mean of plate counts for each of the four methylene blue reduction test classes of milk, data were collected on approximately 1100 samples of raw milk. The number of samples of class four milk was not very large, consequently the data concerning this milk are to be considered as a progress report. The numbers of samples of the first three classes were sufficient to make the results significant.

Samples of raw milk were collected at the weigh vats of several dairies which were being supplied by approximately one thousand farms. Standard plate counts (2) were determined and at the same time the samples were classified according to the methylene blue reduction test (2). The results, after the counts were grouped in a geometric progression fashion since bacteria reproduce in this manner, are given in Table 1. The range of counts was wide for each class, which resulted in considerable overlapping of the range of counts of one class by that of another.

The class two range starts with a count which was one of the most common among the class one milks. Likewise the class three range starts with a count which was one of the most common among the class two milks. The geometric mean of the standard plate counts for class one was found to be 11,500, which on a frequency basis would be included among those counts most frequently encountered for class one milks. The same is true for the geometric mean of class two, 153,900. The geometric mean of classes three and four were 905,200 and 2,796,400 respectively.

The results in Table 1 were obtained from logarithms of the original counts of samples in the four classes. Practically two-thirds of the counts in each class were within one standard deviation of the mean of the loga-

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[2] The author wishes to take this opportunity to express his gratitude to Associate Professor W. D. Baten of the Mathematics Department of Michigan State College for his assistance in interpreting these data.

TABLE 1

Frequencies of plate counts and reduction tests made on raw milk

STANDARD PLATE COUNTS (GROUPS)	METHYLENE BLUE REDUCTION TEST (CLASSES)			
	1 No. of samples	2 No. of samples	3 No. of samples	4 No. of samples
500– 1,000	5			
1,001– 2,000	28			
2,001– 4,000	71			
4,001– 8,000	116	2		
8,001– 16,000	117	14		
16,001– 32,000	61	33		
32,001– 64,000	50	59		
64,001– 128,000	34	84	5	
128,001– 256,000	10	86	15	
256,001– 512,000	8	51	19	1
512,001– 1,024,000		36	32	8
1,024,001– 2,048,000		22	38	7
2,048,001– 4,096,000		10	15	7
4,096,001– 8,192,000		4	9	13
8,192,001–16,384,000				4
Totals	500	401	133	40
Range	1M–500M	5M–6$\overline{M}$	80M–8$\overline{M}$	400M–15$\overline{M}$
* Geometric mean	11,500	153,900	905,200	2,796,400
Mean of logarithms	4.05996	5.18713	5.95674	6.44676
Standard deviation of mean of log.	.02371	.02932	.03910	.06500
** Per cent of counts within 1S.D. of mean	66.2	67.8	67.7	63
** Per cent of counts within 3S.D. of mean	99.7	100	100	100

* This was obtained from the logarithms of the original counts.

** For a normal frequency curve approximately 68 per cent of the sample will be within ± one standard deviation of the mean and approximately 99 per cent within three ± standard deviations.

rithms; more than 99 per cent of the counts in each class were within three standard deviations of the mean of the logarithms, hence no count was discarded.

In a statistical analysis of this type there is always the possibility that the separation between groups or in this case between geometric means may not be significant, which would indicate the possibility of improperly classifying some samples. In order to ascertain the significance of these geometric means found for the different classes of milk, a test for significance (t) was applied.

The means of the logarithms of the counts of bacteria and the standard deviations of these means were obtained for each class. The respective means and their standard deviations for two successive classes were substituted in the following formula to test the significance of the difference of the geometric means. The formula for (t) is

$$t = \frac{\text{Mean log } x_2 - \text{Mean log } x_1}{(\sigma_{\text{mean of log } x_2})^2 + (\sigma_{\text{mean of log } x_1})^2}$$

where x_2 represents the counts of bacteria from one class and x_1 represents the counts from another class, and $\sigma_{\text{mean of log}}$ the respective standard deviation.

If any two geometric means are significantly different, the value of (t) will be in excess of 2.5 (3). The values of (t) for milk classes I and II, II and III, and III and IV were found to be respectively 29.9, 15.7 and 12.2, which means that the geometric means as found are significantly different. These (t) values clearly indicate that the four class geometric means did not come from the same parent population by random sampling and that this test (methylene blue test) will designate the different classes of milk.

SUMMARY

1. The analysis of data of standard plate counts and methylene blue reduction tests of approximately 1100 samples of raw milk is given.

2. The range and frequencies of counts are given for each class as well as the geometric mean and standard deviation.

3. Practically two-thirds of the counts in each class, as determined by the reduction test, were within one standard deviation of the mean of the logarithms and more than 99 per cent of the counts in each class were within three standard deviations of the mean of the logarithms.

4. The geometric means found for the four different classes of milk were found to be significantly different when a test for significance was applied.

5. No attempt has been made in this paper to fix the limits (counts) of the four different classes. This paper shows that the geometric means of the methylene blue classes are significantly different. By examining the standard deviations of the means of the logarithms in Table 1 it is seen that the distributions of the geometric means do not overlap.

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A STUDY OF OILING OFF OF CREAM IN COFFEE*

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The fat emulsion in cream is frequently destabilized to the extent that some of the fat will rise to the surface when the cream is used in coffee. This fat which separates appears on the surface of the coffee in the form of glistening oil droplets or globules which are easily discernible to the coffee drinker. This phenomenon, when it occurs in coffee, is usually referred to by the dairyman as "oiling off."

The oiling off of cream in coffee is objectionable to some consumers of coffee. Buyers for restaurants and hotels frequently place considerable stress on this point when judging coffee cream. They prefer a cream in which the fat emulsion is stable, not only because oiling off is considered objectionable but also because cream with finely dispersed fat phase has a greater effect on the color of the coffee and cream mixture. From these remarks it is obvious that the extent of the oiling off of cream in coffee is of economic importance to the dairyman.

The authors are aware of no references in the literature to the factors causing the oily separation from cream in coffee. However, several studies have appeared which dealt with the changes in the fat emulsion which cause such defects as the cream "plug" in bottled milk or cream, serum separation in bottled cream, and the oily layer on frozen cream when melted. Since the need for research regarding the specific defect of oiling off of cream in coffee was evident, this investigation was carried out.

EXPERIMENTAL METHODS

The facilities of the New England Dairies, Inc., offered an excellent means for the study of this problem. In the several country receiving stations of this corporation various methods of processing cream and a number of types of pasteurizing vats, cream pumps, coolers, etc., were available for investigation. The cream processed in the various country plants could be followed easily as it was shipped to city bottling plants and prepared for delivery.

The need for a suitable test for oiling off was apparent at the beginning of the study. The authors were aware of the hot water and coffee tests applied to cream in the laboratories of several dairy companies. In these

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tests a definite amount of cream is added to 100 cc. of hot water or hot coffee, then the surface of the liquid is examined under the light from a shaded electric light. The globules of oil which separate from the cream will glisten under the light. Using a numerical standard for grading, a badly oiled cream is usually called 4, a desirable cream 0. Such a test though helpful, is subject to the human error of judgment and is not sufficiently accurate to measure small but significant differences.

An attempt was made to develop a microscopic method for measuring the stability of the fat emulsion in cream. However, no definite relationship could be found between the microscopic appearance of the fat globules and their arrangement in diluted cream and the factor of oiling off in coffee.

The test which was developed for use in this investigation has been named the M.S.C. test for oiling off because it was worked out at the Massachusetts State College as a part of this investigation. The test is based on the fact that the globules causing the oily layer are much larger than the normal fat globules of cream and will rise to the surface much more rapidly than the rest of the fat phase. This fat can be centrifuged from a cream-hot water mixture in a regular Babcock skim milk test bottle and can then be measured in the graduated portion of the bottle. The following procedure for the test was adopted after considerable experimentation:

1. One cc. of cream is pipetted into a skim milk test bottle. The skim milk test bottle used should be of the type with the stem extending down into the bottle proper to within one-half inch of the bottom. The stem should have no side hole near the base of the neck as in some styles. The cream sample must be thoroughly mixed and the one cc. taken out immediately so that the oil will not have time to rise to the top of the sample.

2. Wash down the cream in the stem with water at 200° F. and mix by rotating when the water is one-half inch deep in the base of the test bottle, then fill to the shoulder of the bottle which is about one-half inch from the base of the neck.

3. Centrifuge in a heated Babcock machine for positively no longer than ten seconds after the machine has reached speed. If the sample is whirled longer than ten seconds a plug will form which is hard to put back into suspension and the particles will clog the neck.

4. Remove the bottle from the centrifuge and tap the side of the bottle to break the very thin film which has formed. Do not agitate.

5. Now add water again at 200° F. and bring the column well up into the neck.

6. Centrifuge for five minutes.

7. Read the oil layer as soon as removed from the hot centrifuge. Each small division of the graduated scale may be read as 1.

The operator of the M.S.C. test should follow the directions closely. Some difficulty was experienced at first with the test in securing duplicate

results. However, after sufficient experience it became evident that strict adherence to the routine of the test as well as thorough mixing of the cream and quick pipetting of the sample are all very essential if accurate readings are to result.

No attempt has been made to convert the readings to a quantitative expression of the amount of oil separating from the cream. For that reason it is suggested that the readings be given in whole numbers, with the smallest division on the neck of the bottle as 1.

Obviously, the fat which is separated in the test should not be read quantitatively since the one cc. of cream used in the determination is not weighed and the creams being tested vary in fat content. Measuring the cream is more satisfactory than weighing because (1) the slight variations in weight from sample to sample produce no noticeable variations in the results and (2) speed in taking the sample is essential. The sample being tested should be stirred thoroughly and 1 cc. pipetted from it promptly. Our experience indicates that the separated oil droplets will tend to rise rapidly towards the surface of the cream sample.

This test is now being used satisfactorily in commercial laboratories in Boston. Creams which produce readings of 1 division or less do not show an oily separation when used in coffee; creams with a reading of two divisions are usually considered acceptable while those with a reading of three or more oil off in coffee to a noticeable degree and are considered unsatisfactory by many cream buyers.

EXPERIMENTAL RESULTS

The results given in Tables 1 to 5 show the effect of a number of processes and types of equipment on the stability of the fat emulsion in cream.

In Table 1 the effects of several receiving plant processes are given. When approximately 40 per cent of the milk received was partially frozen, the test on the resultant cream was 3 and, as stated previously such cream is unsatisfactory for use in coffee. Part 2 of Table 1 shows that the test of the milk being separated, so long as the milk is normal in other respects, has a normal reading of 1 or less. However, the separation temperature is important (Table 1, Part 3). As the separating temperature is increased, the tendency to oil off increases. Temperatures of 85° F. and 90° F. gave normal results; a definite increase appeared at 120° F.; and 140° F. (with mechanical agitation in the preheater) damaged the emulsion to the extent that the cream tested 3, hence would oil off noticeably in coffee.

The fat content of the separated cream evidently does not influence the stability of the fat emulsion unless the cream contains 50 per cent butter fat or more (Table 1, Part 4). The results show that an increase in the reading for oiling off was evident with 50 per cent cream, but not for cream of lower fat content.

TABLE 1

The effect of some properties of milk and certain receiving plant processes on the oiling off factor in the resultant cream

FACTOR OBSERVED	TREATMENT OF MILK OR CREAM	AMOUNT OF OILING OFF (M.S.C. TEST READING)	
1. Effect of freezing milk			
A. Normal milk	.....	.5 divisions	
B. Partially frozen milk	40% of milks partly frozen when received	3.0 "	
2. Test of milk separated			
A. 4.7% milk	Separated at 90° F., cooled, cream raw	.5 divisions	
B. 4.0% "		.5 "	
3. Separating Temperatures			
A. 85° F.	Internal tubular preheater used	.5 divisions	
B. 90° F.	" " " "	.5 "	
C. 120° F.	" " " "	1.0 "	
D. 140° F.	Barrel heater, with paddle agitator used	3.0 "	
4. Test of separated cream			
A. 30% cream	Cream separated at 90° F., not pasteurized	.5 divisions	
B. 40% "		.5 "	
C. 45% "		.5 "	
D. 50% "		1.0 "	
5. Temperature of cream when standardized		Reading Before Stand.	After Stand.
A. 90° F.	Creams standardized to 40% fat	.5	.5
B. 120° F.		1.0	1.0
C. 135° F.		1.0	1.0
D. 145° F.		1.0	1.0
6. Standardization Product			
A. 4.0% milk	The tests were run on the cream before and	1.0	1.0
B. Skimmed milk	after standardization at 120° F.	1.0	1.0

TABLE 2

Effect of handling the cream in the pasteurization vat on the oiling off factor of the cream

FACTOR OBSERVED AND TREATMENT OF CREAM	AMOUNT OF OILING OFF IN CREAM (M.S.C. TEST READING)	
	RAW CREAM	BEFORE 30′ HOLDING PERIOD
1. Treatment of cream in vat		
A. Held at 90° F. until vat is full before heating is begun	.5 division	1.0 divisions
B. Heating of cream done while vat fills	.5 "	1.0 "
2. Prolonged holding of cream prior to the pasteurization process		
A. Cream cooled to 50° F., held 3 hrs. without agitation	.5 "	1.0 "
B. Cream held at 90° F. for 3 hrs. without agitation	.5 "	1.0 "
C. Cream held at 135° F. for 3 hrs. without agitation	.5 "	3.0 "
D. Cream held at 135° F. for 4 hrs. with agitation	.5 "	4.0 "
	Before holding	After holding
3. Time cream is held at the pasteurization temperature		
A. Cream held at 145° F. for 30 min.	1.0 division	1.0 divisions
B. " " " " " " 40 "	1.0 "	2.0 "
C. " " " " " " 50 "	1.0 "	2.0 "
D. " " " " " " 70 "	1.0 "	3.0 "

4. Amount of cream in vat	Raw cream	Before holding	After 30′ holding	After cooling
A. Spray vat, 800 qt. capacity, filled with cream	.5	1.0	1.0	1.0
B. Spray vat, 800 qt. capacity, one-fourth full	.5	2.0	4.0	4.0

In the receiving stations where these observations were made, the separators are adjusted to separate cream testing a little higher than 40 per cent butter fat. The cream is then standardized to a fat content of 40 per cent prior to pasteurization. The results in Part 5 of Table 1 show that the cream can be standardized at any temperature up to the pasteurization temperature without any marked tendency towards oiling off, although slightly better results were secured when standardization was done while the cream was below 120° F.

Either skimmed milk or whole milk may be used for standardization. It is evident from Part 6 of Table 1 that neither of these products increases the tendency to oil off when used to reduce the fat content of high testing cream.

Various factors involved in the pasteurization of cream, which might affect the stability of the fat emulsion, are summarized in Tables 2 and 3.

In some receiving stations cream is gradually heated to the pasteurization temperature while the vat fills with cream. In other instances the cream is not heated until the vat is filled. In either case the cream is not agitated while the vat fills. Part 1 of Table 2 shows that the two methods have the same effect on the tendency to oil off. An increase in the reading to a value of 1 must be considered as a normal increase which cannot be avoided. Raw cream showing little or no oil separation will have a reading of 1 when brought to the pasteurization temperature.

From Part 2 of Table 2 it is evident that freshly separated cream can be held without agitation for at least 3 hours, either at 50° F. or 90° F. without decreasing the stability of the fat emulsion. However, it is very evident that the cream should not be held at 135° F. for any length of time for with this treatment a test for oiling off gave readings of 3 and 4 points. The holding temperature of cream before pasteurization is of significance because in some receiving stations considerable time elapses before the vat is filled with cream and pasteurization is begun. In some plants the cream is cooled to 50° F., and in others it is held at the separation temperature of 90° F. Either practice is satisfactory. But in plants where the cream is held a few degrees below the pasteurization temperature for some time the stability of the fat emulsion is evidently damaged.

The length of the holding period in pasteurization is also of significance (Table 2, Part 3). As is evident from these data, no increase in oiling off occurs during the 30 minute holding period at 145° F. but an increase of only 10 minutes in the holding period increased the reading 1 point, a significant amount. When the holding period was increased to 70 minutes, the cream oiled off considerably in coffee, as the reading of 3 indicates.

The fullness of the vat is also of importance. When a "spray vat" was filled with cream the pasteurization process did not injure the fat emulsion. However, when the same vat was less than half full of cream, the pasteuriza-

TABLE 3

Effect of type of vat, temperature of heating medium, type of agitation and method of pumping cream to cooler on the oiling off factor in cream

FACTOR OBSERVED AND TREATMENT OF CREAM			AMOUNT OF OILING OFF (M.S.C. TEST READING)			
			Raw cream	Before holding	After holding	After cooling
1. *Type of vat*	*Temp. heating medium*	*Type of agitation*				
A. Stainless steel spray	160° F.	Slow speed paddle	.5	1.0		
B. Stainless steel spray	190	Slow speed paddle	.5	3.0		
C. Glass lined steel	160	Slow propellor	.5	1.0		
D. Glass lined steel	212	Fast propellor	.5	4.0		
E. Coil vat	160	Slow speed coil	.5	3.0		
F. Coil vat	210	Slow speed coil	.5	3.0		
2. Agitation during holding period of 30 min. Cream at 145° F.						
A. Stainless steel spray vat, slow paddle agitation			.5	1.0	1.0	
" " " " no agitation			.5	1.0	1.0	
B. Glass lined vat with slow propellor agitation			.5	1.0	1.0	
" " " " no " "			.5	1.0	1.0	
" " " " fast " "			.5	3.0	5.0	
3. Temperature of pasteurization.						
A. Cream pasteurized at 143° F. in stainless steel vat, no agitation during holding period.			.5	1.0	1.0	
B. Same as A, except at 150° F. pasteurization temperature.			.5	2.0	2.0	
C. Same as A, except at 160° F. pasteurization temperature.			.5	2.0	2.0	
4. Pumping cream from pasteurizer to surface cooler by						
A. Proper size centrifugal pump.					2.0	2.0
B. Oversize centrifugal pump.					3.0	4.0
C. Steam piston pump.					1.0	1.0
D. Steam piston pump.					3.0	2.0

tion process caused the test to increase to 4 points and the cream oiled badly in coffee.

The data given in Table 3 indicate that the type of vat, temperature of heating medium, and type of agitation all affect the stability of the fat emulsion in cream. Stainless steel spray vats with slow-speed paddles and glass-lined steel vats with slow-speed propellor agitators proved satisfactory when used with a heating medium of 160° F. However, coil vats increased the reading to 3, regardless of the temperature of the heating medium. Hence it is evident that this type of vat should not be used for pasteurizing cream if oiling off of the cream in coffee is to be prevented.

Good results were secured in spray vats and glass-lined steel vats, with slow agitation and a heating medium of 160° F. However, if the heating medium was increased to 190° F., or the speed of the agitator increased, then the cream was made less stable against oily separation in coffee (Table 3, Part 1).

Part 2 of Table 3 shows that holding cream during pasteurization in spray or glass-lined steel vats, either with slow-speed agitation or with no agitation at all produces identical results. Therefore agitation of the cream during the holding period can be practiced, if desired, with the knowledge that no harm to the fat emulsion is being done. However, the tendency to oil off was noticeably increased when the propellor agitator in the glass-lined steel vat was shifted to high speed, both during the heating period as well as during the holding period.

Part 3 of Table 3 shows that heating the cream to a pasteurization temperature of 150° and 160° F. causes an increase in the test for oiling off to a reading of 2. The figures show that there is no increase in free oil during the holding period but rather the increase occurs during the heating process. The longer heating period, with necessarily a longer period of agitation, undoubtedly causes this increase.

The type of pump used to transfer cream from the pasteurizing vat to the cooler was also studied (Part 4 of Table 3). The data show that no increase in the oiling off test reading occurred when a proper size centrifugal pump was used but when an oversize pump was used evidently more agitation occurred, which caused the reading to increase. The steam piston pump also proved satisfactory. In fact the results indicate that with cream which already has a high reading, the piston pump tends to re-emulsify the separated oil. This is evidently the case for in test D of Part 4, Table 3, the test for oiling off was actually lowered a point while the cream passed through a steam-driven piston pump.

Examination of Table 4 reveals that no increase in the tendency to oil off occurs during cooling of cream if agitation within the temperature range of 40° to 100° F. is prevented. Complete cooling of cream over a surface cooler or cabinet cooler caused no increase in the test; this is also true with

partial cooling (to 120° F.) in spray vats and stainless steel vats, with slow agitation, then followed by the use of a surface cooler. However, when cream was cooled to 50° F. in a spray vat the test for oiling off showed a 2 point increase. Complete cooling in a coil vat also caused a marked increase in the free oil content of the cream.

From Part 2 of Table 4 it is evident that the stability of the fat emulsion is not affected by cooling cream over the surface cooler to 40° rather than 50° F. However, when some cream froze to the lower coil of the surface cooler the results show a considerable increase in free oil. The authors have found this to be a common cause of oiling off of cream in coffee.

The aging of cream for 24 or 48 hours after pasteurization had no effect on the stability of the fat emulsion (Part 3, Table 4).

TABLE 4

Effect of methods of cooling cream, aging, and shipment on the oiling off factor in the cream

TREATMENT OF CREAM	EXTENT OF OILING OFF (M.S.C. TEST READING)	
	After holding	After cooling
1. Method of cooling cream.		
A. Large surface cooler.	1.0	1.0
B. Cabinet cooler.	1.0	1.0
C. Partial cooling in spray vat then pumping over surface cooler.	1.0	1.0
D. Complete cooling in spray vat.	4.0	6.0
E. Partial cooling in glass lined vat with fast propellor agitator.	3.0	3.0
F. Partial cooling in glass lined vat with slow propellor agitator.	1.0	1.0
G. Complete cooling in coil vat.	5.0	7.0
2. Temperature to which cream is cooled.		
A. Drawn from surface cooler at 40° F.	1.0	1.0
B. Drawn from surface cooler at 50° F.	1.0	1.0
C. Some freezing on brine section of surface cooler.	3.0	6.0
3. Aging cream at 38° F.		After aging
A. No hours.		1.0
B. 24 "		1.0
C. 48 "		1.0
4. Transportation of cream at 40° F.	Before shipment	After shipment
A. Full can of cream shipped by train.	1.0	1.0
B. Full can of cream shipped by truck.	1.0	1.0
C. Half-full can of cream shipped by train.	1.0	2.0
D. Half-full can of cream shipped by truck.	1.0	4.0

Transportation of cream at approximately 40° F., either by train or truck, did not decrease the stability of the fat emulsion of the cream providing the cans were filled. However, in partially filled cans enough agitation occurred to increase the test reading for oiling off by one point when the cream was shipped by rail and by 3 points when transported by truck (Part 4, Table 4). The handling distance in each instance was about 140 miles.

Some milk distributors purchase sweet cream for bottling and standardize it merely by mixing it with cold pasteurized milk. When cream with a stable fat emulsion is treated in this way, no damage is done and the resultant standardized creams do not oil off (Part 1, Table 5). However, when cream testing 40 per cent butter fat, which oiled off badly, was reduced by this method to 30 and 20 per cent fat the lower testing creams also oiled off to the same extent as the original cream.

Cream which has been destabilized by any of the previously discussed factors can be made entirely satisfactory by homogenization (Part 2, Table 5). The data show that cream with a reading of 4, when tested for the degree of oiling off, was made entirely stable by a homogenization pressure of 500 pounds per square inch. A pressure of 300 pounds is practically as efficient. Some of the unstable cream was also passed through a colloid mill at a pressure of 150 pounds per square inch and through a "hand emulsor" made for home use. Both of these machines partially re-emulsified the free oil but were less satisfactory than the homogenizer.

Data have been omitted for the sake of brevity concerning several points which were studied. Varying the mineral content of cream by the addition of sodium citrate or lime water produced no perceptible effect on the oiling off factor in cream. This also proved true when gelatin was added to cream and when the acidity of the cream varied within the limits encountered in commercial practice.

The method used in making coffee seems to have no effect on the stability of the fat in the cream added to the coffee. Whether coffee was poured into the cup before the cream or *vice versa* had no effect on the appearance of oil on the surface of the coffee. A few different brands of coffee were used but none seemed to increase the oily layer appearing on the cream and coffee mixture.

Cream which was allowed to stand at room temperature for several hours lost its stability and oiled off in coffee. However, cream which was kept in a refrigerator except at meal time did not deteriorate appreciably after three days in respect to oiling off when used in coffee.

SUMMARY AND CONCLUSIONS

1. A suitable test for measuring the extent to which cream will oil off in coffee has been developed and is presented as a part of this paper. Creams

TABLE 5

The effect of standardization and homogenization on the oiling off factor in cream

TREATMENT OF CREAM	AMOUNT OF OILING OFF (M.S.C. TEST READING)
1. Standardization of cream	
A. Pasteurized 40% cream	1.0
A_1 Same as A but reduced to 30% cream, using whole milk	1.0
A_2 Same as A but reduced to 20% cream, using whole milk	1.0
B. Pasteurized 40% cream	3.0
B_1 Same as B but reduced to 30% cream, using whole milk	3.0
B_2 Same as B but reduced to 20% cream, using whole milk	3.0
2. Re-emulsification of fat in cream which oils off	
A. Control—cream pasteurized and cooled	4.0
B. Same cream as the control, except homogenized at 500 lbs. pressure	0
C. " " " " " " " " 1000 " "	0
D. " " " " " " " " 1500 " "	0
E. " " " " " " milled in colloid mill at 150 lbs. pressure	.5
F. Same cream as the control, except passed through "hand emulsor"	2.0

yielding a reading of 3 or above with this test will show a noticeable oily separation when used in coffee.

2. A number of factors have been studied which affect the stability of the fat emulsion in cream and have a bearing on the problem of oiling off of cream in coffee. The more important factors are summarized as follows:

a. When milk which has been partially frozen is separated, the resultant cream will produce an oily separation in coffee.

b. As separating temperatures are increased above 90° F. the fat emulsion in the cream becomes progressively less stable when the cream is used in coffee. Mechanical agitation of the milk during preheating prior to separation proved undesirable. Separating milk into cream of more than 45 per cent fat also caused a decrease in the stability of the fat emulsion.

c. The temperature of cream when standardized in fat content by the addition of whole or skimmed milk has no effect on the fat emulsion.

d. If vats are filled rapidly with cream and heated slowly to 145° F. a minimal increase in the tendency to oil off results. When vats fill slowly equally desirable results are secured if the cream is held below 90° F. and not agitated. Agitation and the heating of cream to a temperature near that of pasteuriaztion are undesirable practices and cause the oily separation.

e. Prolonged holding of cream at the pasteurization temperature increases the amount of destabilized fat in cream. The partial filling of pasteurization vats causes similar undesirable results. Stainless steel or glass-lined steel vats with slow propellor or paddle agitation, and a heating medium of 160° F., produced satisfactory results. However, an increase in the speed of agitation or in the temperature of the heating medium, in these same vats, increased the oiling off tendency. Coil vats, regardless of the temperature of the heating medium destabilized the fat emulsion. Heating cream to pasteurization temperatures higher than 145° F. increased the test for oil separation slightly. Whether cream is agitated slowly or not at all during the holding period apparently made no difference, but rapid agitation caused oiling off.

f. Pumping cream from pasteurizer to cooler by proper size centrifugal pumps had no effect on the fat emulsion, while the use of oversize pumps destabilized the fat to some extent. Steam piston pumps do not affect the fat emulsion adversely; in fact, with cream which oiled off badly, the piston pumps partially re-emulsified the fat which had separated.

g. Cooling cream in the pasteurizing vat was found to be very undesirable regardless of the type of agitation employed. The final temperature to which cream is cooled over a surface cooler does not affect the fat emulsion so long as freezing on the cooler does not occur. Freezing of

cream to the cooler was found to be one of the most serious causes of oiling off of cream in coffee.

h. Aging cream (without agitation) at low temperature caused no change in the stability of the fat emulsion. Shipment at low temperature also had no effect unless the cans were partially filled, thus allowing for agitation which proved harmful.

i. Reducing the fat content of pasteurized cream by the addition of milk or skim milk had no effect on the tendency of the cream to oil off.

j. Cream which had been improperly handled, so that droplets of oil would separate from it if used in coffee, was made entirely stable by homogenization of the cream at the pasteurization temperature. A pressure of 500 pounds was used. Attempts to re-emulsify the fat by the use of a colloid mill and manually operated emulsor were partially successful.

k. Storage of cream for three days in a household refrigerator did not increase the tendency to oil off when the cream was used in coffee.

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United States Department of Agriculture

ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

BACTERIOLOGY

436. B. W. Hammer Panegyric, by His Former Students. 1937, p. 250. Published by the Collegiate Press, Inc., Ames, Iowa. Price $2.50.

This book was prepared by former students of B. W. Hammer as a tribute to him after 25 years of service at Iowa State College. The book is divided into two sections, the first one consists of 18 pages devoted to tributes to Dr. Hammer, and the second part consists of articles each written by a former student on various phases of the bacteriology and chemistry of dairy products. These individual articles deal principally with very recent investigations so that they are particularly valuable as giving new knowledge in the different subjects.

The best idea of the scope of the book may be obtained from a listing of the titles of chapters which are as follows:

Further observations on the quantitative changes in the microflora of cream and butter during manufacture, storage and shipment.

Churn contamination as a source of yeasts and molds in butter.

A comparison of media for determining the total bacterial count of butter.

Some observations on the yeast and mold count of salted butter made from sour cream.

The influence of filtration of inoculated wash water on bacterial count and keeping qualities of butter.

A method for the microscopic examination of butter.

The influence of starter on the flavor of butter.

The influence of various methods of neutralizing cream on the quality of fresh and stored butter.

A comparative study of Mississippi and Minnesota butter from the standpoints of certain fat constants and heat resistance.

The manufacture of high-scoring butter.

The effect of certain pencillia on the volatile acidity and the flavor of Iowa Blue cheese (Roquefort type).

Methods used to increase blue mold growth in cheese.

The effect of *Pencillium roqueforti* on some lower fatty acids.

The influence of certain bacteria on the ripening of cheddar cheese made from pasteurized milk.

Curing small units of American cheese in liquid paraffin.

A study of some of the physical changes involved in the rennet coagulation of milk and the subsequent firming of the curd.

Physical and chemical effects of homogenization of milk.

Distribution of bacteria in a quart bottle of whole milk held at 0° C.

Influence of growth temperature on the thermal resistance of some aerobic, spore-forming bacteria from evaporated milk.

The influence of growth temperature and age on the thermal resistance of milk cultures of *Streptococcus lactis*.

The limitations of significance of some of the methods of analyzing ice cream.

Studies on *Bacillus coagulans*.

Bacteria of the Escherichia-Aerobacter group in dairy products.

Observations on *Alcaligenes lipolyticus*.

Studies of lactobacillus cultures that actively coagulate milk.

Determination of acetylmethylcarbinol and diacetyl in dairy products.

A study of the distribution of strains of *Streptococcus lactis* which are sensitive to a filterable inhibitory principle from slow starters. A.C.D.

437. Milk Examination. A Comparison of the Plate Count and Reductase Test. J. S. Faulds. Lancet. *232*, p. 949, April 17, 1937.

To ascertain how far the results of the methylene-blue reductase test and the plate count are in agreement, the author examined 1500 samples by both methods. He found that 22.3 per cent of the samples passed one test but not the other, the inconsistency being greatest in June and July during warm weather. A total of 5.3 per cent passed the reductase test, but failed by the count and coli estimation, while 17 per cent passed the count and coli standard, but failed by the reduction test. The discrepancy was greatest in milks of higher temperatures.

The author concludes that the methylene-blue reductase test is much simpler to apply than the plate count and yields 75 per cent of comparable results. It is, on the whole, more stringent in warm weather and less stringent in cold weather. Arguments in favor of the economy of this test are weak if the cost of collection is taken into account. J.A.T.

438. Relation of Bovine Mastitis to Human Disease. Paul B. Brooks and Walter Von D. Tiedeman, N. Y. State Dept. of Health, Albany, N. Y. Am. J. Pub. Health *27*, 4, p. 334, 1937.

The authors believe that the organisms responsible for cases of mastitis associated with outbreaks of septic sore throat and scarlet fever are always of human origin, *i.e.*, persons, usually milkers, with infected throats or occasionally wounds, even though it has not always been possible to find the individual.

The possible relation of mastitis to outbreaks of gastroenteritis and the evidence that organisms commonly present in mastitis may invade human tissues are discussed. The general control of mastitis, if feasible, probably should be considered primarily an economic problem of the dairy industry since only occasional cases of mastitis are responsible for serious epidemics of disease. Pasteurization destroys all of these organisms and probably their toxins as well, but when milk is being sold without pasteurization, all cases

of mastitis should be regarded as potentially dangerous and milk from any cow suffering from the disease carefully excluded. M.W.Y.

439. Control of Septic Sore Throat. G. J. HUCKER, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Am. J. Pub. Health *27*, 4, p. 313, 1937.

Reasons for the difficulty in determining the true incidence of scarlet fever and septic sore throat in the United States are presented. Scarlet fever is primarily a contact disease while septic sore throat usually spreads from a common source of infection and not through contact. Septic sore throat in the main is traced to raw milk supplies. The cause and epidemiology of septic sore throat and the need for more reliable statistics on scarlet fever and septic sore throat infections are discussed. M.W.Y.

440. The Identification of Streptococcus of Mastitis in Routine Milk Samples. W. L. WILLIAMS, Univ. of Louisville, Louisville, Ky. Am. J. Pub. Health *27*, 5, p. 453, 1937.

An abstract of a paper read at the annual meeting in 1936. About 90 per cent of streptococcus strains causing bovine mastitis can be classed as *Streptococcus agalactiae*. Cultural characteristics are described for the strains which were isolated. M.W.Y.

441. Rapid Detection of B. Tuberculosis in Milk. MARY L. COWAN MAITLAND. Lancet 232, p. 1297, May 29, 1937.

A rapid method for direct microscopic examination of milk for tubercle bacilli has been tried on quarter samples from 950 cows and all results checked with the results of biological tests, except in one instance.

The procedure outlined is to spin the milk for 3 minutes at 2500 r.p.m., make a film from the deposit, dry in air and after half an hour fix with flame, cool, place in alcohol and ether (equal parts) for 15 minutes, wash with ether, stain with steaming carbol-fuchsin for 8 minutes, wash in water, decolorize in 3 per cent HCl in alcohol for 3 minutes, wash in water, decolorize in fresh acid-alcohol for 3 minutes, wash, counterstain with Löffler's methylene-blue for 2 minutes, wash well with water, dry, and examine under low power objective ($\frac{2}{3}$ in.) of microscope. J.A.T.

442. Report of Committee on Communical Diseases Affecting Man. JOHN G. HARDENBERGH, 23rd Ann. Report of Intern. Assoc. of Dairy and Milk Inspectors, Oct. 1934, p. 69.

This report covers statistical data on milk-borne diseases for 1933 and types of milk supply involved.

Factors necessary for a safe milk supply are discussed. L.H.B.

443. The Gas Requirements of Mold.—I. A Preliminary Report on the Gas Requirements of Pencillium Roqueforti (Various Strains of Blue Mold from Cheese). N. S. GOLDING, Div. of Dairy Hus-

bandry, Agr. Exp. Sta., State College of Washington, Pullman, Washington. JOURNAL OF DAIRY SCIENCE 20, 6, p. 319, June 1937.

The presence of carbon dioxide inhibited the growth of *Pencillium roqueforti*. A.C.D.

BUTTER

444. Methods for Making and Significance of Cream and Butter Sediment Test. E. H. PARFITT, Dairy Dept., Purdue University, Lafayette, Indiana. Am. J. Pub. Health *27*, 4 p. 341, 1937.

Methods for determining sediment in cream and butter are outlined. The sediment in cream, like the sediment in milk, indicates carelessness on the part of the producer. Classification of sediment from butter is discussed. M.W.Y.

445. A Modified Test for Salt in Butter. L. A. BRYANT, O. A. C. GUELPH. Can. Dairy and Ice Cream J. *16*, 6, p. 19, June 1937.

The author offers a modification of the standard salt test for butter. In the modified test two drops of dichloro-fluorescein solution instead of two drops of potassium chromate indicator solution is added to the 25 cc. of salt solution. This indicator is recommended in place of potassium chromate since it gives a much sharper end point. J.C.H.

446. Quantitative Determination of Lactic Acid in Butter. H. C. TROY AND PAUL F. SHARP, Cornell Univ., Agr., Exp. Sta, Ithaca, N. Y. Memoir 202, March 1937.

Three methods for the determination of lactic acid in butter were investigated. These methods all depend on the oxidation of lactic acid to acetaldehyde, the removal of the acetaldehyde from the oxidation mixture, the collection of the acetaldehyde in sodium-bisulfite solution, and the titration of the bound bisulfite with iodine. The methods differ in the amount of time required and the completeness of the removal of interfering substances.

Three procedures were studied. Troy and Sharp extraction method, Whittier and Trimble direct-oxidation method, and the direct-precipitation method. The last method is recommended.

It was found that after the butter is made, little increase in its lactic-acid content occurs when it is held even at fairly warm temperatures, and no increase at all occurs when it is held in cold storage.

The determination of lactic acid in butter serves as an indication of the lactic-acid content of the cream from which the butter was made, and is useful, therefore, in classifying butter on this basis. The lactic-acid contents of a number of samples of commercial butter are given.

The determination of lactic acid in the butter and in the buttermilk enables one to determine, by a simple calculation, the amount of buttermilk

remaining in the butter, and, from a knowledge of the water in the butter, to determine the efficiency of washing.

The determination of the lactic-acid content of the butter enables one to estimate the degree of souring of the cream from which the butter was made, and the determination of the pH indicates whether the cream was neutralized prior to churning. E.S.G.

Other Abstracts of interest are numbers 436, 452, 453, and 455.

CHEESE

447. Cheese Mites and Their Control. G. G. DUNSTAN, O. A. C., GUELPH. Can. Dairy and Ice Cream J. *16*, 6, p. 26, June 1937.

This paper presents briefly the results of an interesting study of cheese mites, including species responsible, life history, nature of injury to cheese, method of distribution, conditions favoring mites and methods of control.

A study of fumigants has been made as one method of control. Requisites of a good fumigant for cheese and precautions in fumigation are given.

The author states, that, "Of the fumigants tested only two were found satisfactory: (a) methyl bromide 6.8 per cent plus carbon dioxide 93.2 per cent, and (b) ethylene oxide 1 part plus carbon dioxide 9 parts." J.C.H.

448. Wrappers for Processed Cheese. HUGH L. TEMPLETON AND H. H. SOMMER, Dept. Dairy Industry, Univ. of Wis., Madison, Wisconsin. JOURNAL OF DAIRY SCIENCE *20*, 5, p. 231, May 1937.

The various factors of importance in the selection of cheese wrappers are given and the data presented indicated tinfoil to be as satisfactory as any wrapper available now. A.C.D.

449. The Microbiological Flora on the Surface of Limburger Cheeses. C. C. KELLY, N. Y. State Agr. Exp. Sta., Geneva, N. Y. JOURNAL DAIRY SCIENCE *20*, 5, p. 239, May 1937.

The bacterial flora of Limburger cheese were predominantly yeasts for several days and later was chiefly *Bacterium linens*. A.C.D.

450. Observations on the Salting of Brick Cheese. E. L. BYERS AND WALTER V. PRICE, Univ. of Wis., Madison, Wisconsin. JOURNAL OF DAIRY SCIENCE *20*, 6, p. 307, June 1937.

Although salt penetrated quickly into brick cheese a uniform distribution was not secured for 8 weeks. A.C.D.

Other Abstracts of interest are numbers 436, 438, 439, and 443.

CHEMISTRY

451. The Production of Milk of Abnormal Composition by Animals Free From Udder Streptococci. E. G. HASTINGS AND B. A. BEACH, Univ. of Wisconsin. J. Agr. Research *54*, 3, p. 199, 1937.

A herd of 31 one-year-old Holstein-Friesian heifers, free of tuberculosis and Bang's disease, was assembled on a farm on which there had been no cattle for 8 months and where the stables had been disinfected after removal of the previous herd. The herd remained isolated from other animals except for the addition of a herd bull. After the heifers freshened at about 2 years of age their milk was studied periodically for abnormalities which might indicate mastitis. Three thousand samples of foremilk from separate quarters were analyzed for chlorine, catalase, and pH value. Each sample was cultured on glucose agar and in milk. Additional samples of foremilk and of the entire product of each quarter from animals producing abnormal milk were examined in greater detail as to composition and bacterial content.

The results show that on any periodic monthly examination from 4 to 16 of the heifers would have been adjudged as affected with chronic mastitis on the basis of the three criteria used. However, no streptococci were found in any sample, the condition apparently being related to higher numbers of the usual types of bacteria found in the udder. The observations did not indicate that the cause of the condition passes from animal to animal. In some of the animals the abnormality in a quarter was present at the beginning of the lactation period and persisted throughout the period. In other cases the abnormality appeared at some time during the lactation period and continued to the end thereof, and in still other cases it disappeared. One or more samples of milk which satisfied the criteria of abnormality used, namely, a pH value above 6.8, a chlorine value above 0.15 per cent, and a catalase value in excess of 50 per cent, were obtained from 23 of the 31 cows. The detailed study of the records indicates that 17 of the animals may be considered as normal; 14 as abnormal. L.M.T.

452. The Higher Saturated Fatty Acids of Butter Fat. GEORGE E. HELZ AND A. W. BOSWORTH, Lab. of Physiol., Ohio State Univ., Columbus, Ohio. J. Biol. Chem. *116*, p. 203, 1936.

Extended fractionation of the methyl esters of the fatty acids from 360 pounds of butter was made to ascertain further the character of the fatty acids of high molecular weight. The undistilled residue not distilling below 215° at 15 mm. was prepared and fractionated at 5 mm. pressure and up to 245°. In these fractions was indicated presence of saturated acids from C_{18} (mol. wt. 284.4) to C_{24} (mol. wt. 368.5). Presence of behemic acid in significant quantities was indicated. In the residue not distilling below 245° was recovered hexacosanoic (cerotic) acid. This crystallizes as nacreous crystalline plates from acetone and has a melting point of 80.5° C. The methyl ester distills at 286° at 15 $\pm$ 0.1 mm. pressure and at 261° at 5 $\pm$ 0.1 mm. pressure. The melting point of the methyl ester is 62°. K.G.W.

453. The Carotene of Milk Fat (Butter). A. E. Gillam and M. S. El Ridi, Chemistry Dept., Victoria Univ., Manchester, England. Biochem. J. *31*, p. 251, 1937.

With the discovery that carotene possesses growth promoting property (vitamin A activity) its presence in butter became important from a nutritional aspect, while later discovery that the pigment occurs in several isomeric forms having different vitamin A activities at once raised question as to which form was present in butterfat. The small amount of carotene in butter (0.2–2.0 mg. per 100 g. of fat) has made its isolation and examination difficult. In this investigation analytically pure butter carotene has been isolated from a mixed sample of colostrum and ordinary milk fat, and shown by analysis, m.p., absorption spectra and optical rotation to be pure beta carotene. The data show that for the sample of fat examined, alpha carotene is either absent, or is present in negligibly small amounts (less than 0.3 per cent of the total carotene). K.G.W.

454. The Determination of Galactose by the Method of Hagedorn and Jensen. Ernest F. Gale, Biochem. Lab., Cambridge, England. Biochem J. *31*, p. 234, 1937.

A method is described for the estimation of amounts of galactose between 0.1 and 0.4 mg. The method is not reliable for lesser amounts. Bacteria of fermentation mixtures are coagulated by heating with zinc sulphate solution and are filtered off before the estimation of the sugar. The method applies the procedure involving reduction of ferricyanide by sugar, the excess ferricyanide being estimated iodometrically. K.G.W.

455. The Oxidation of Butterfat. II. The Composition of the Fat in Relation to Its Susceptibility Toward Oxidation. V. C. Stebnitz and H. H. Sommer, Dept. of Dairy Industry, Univ. of Wis., Madison, Wisconsin. Journal of Dairy Science *20*, 5, p. 265, May 1937.

Among other conditions it was stated that it is linoleic rather than oleic acid in butter fat which governs the rate of oxidation. A.C.D.

456. Lipaemia and Milk Fat Secretion in the Ruminant. Francis X. Aylward, Janet H. Blackwood and James A. Smith. Hannah Dairy Research Inst. Kirkland, Ayr. Scotland. Biochem. J. *31*, 30, 1937.

Iodized fats, the proprietary names of which were "Radiopol" and "Lipiodol," were fed cows in an experiment designed to elucidate (1) the existence and nature of alimentary lipaemia in the bovine, (2) the rate at which fatty acids fed as glycerides enter the blood lipins and their relative distribution therein; and (3) the existence of a relationship between the level of a particular fatty acid in the blood and its secretion in the milk.

Fatty acids of the diet passed into the fat components of the blood enriching them in accordance with the composition of the dietary fat itself, the whole process following the course of a smooth lipaemia curve attaining a maximum in the case of the ruminant in 2 days and returning almost to normal at about the 5th day. For the iodized fat administered in the experiments, the amount secreted in the milk appears to be directly related to the amount in the blood. The proportion of the dietary fat carried in the blood as phosphatide was observed to be less than that carried as non-phosphatide, from which it would appear probable that phosphatide is not the chief means of transport in the blood, nor is it the precursor of milk fat, the latter rôle appearing to belong either to the glycerides or the cholesterol esters or both. K.G.W.

CONCENTRATED AND DRY MILK

Abstracts of interest are numbers 436, 437, 451, 457, 458, 460, 472, 473, 475, 476, and 477.

FOOD VALUE

457. Prophylaxis of Rickets in Infants with Irradiated Evaporated Milk. J. T. Davidson, K. K. Merritt, and S. S. Chipman. Am. J. Diseases of Children *53*, 1, p. 1, 1937.

Eighteen premature and fifteen full term infants were given irradiated evaporated milk during the first six months of life to determine degree of protection afforded.

Judged by roentgenograms, none of the premature infants was entirely free from rickets, six showed slight rickets, nine showed moderate rickets and three showed marked rickets.

Full term infants, with one exception, were almost completely protected against all but the slightest roentgenologic rickets. This was in marked contrast to the more severe degree of rickets observed in the rapidly growing premature infants. W.H.R.

458. Comparison of the Anti-Rachitic Effects on Human Beings of Vitamin D from Different Sources. T. G. H. Drake. Am. J. Diseases of Children *53*, 3, p. 754, 1934.

No evidence was obtained of any difference in the antirachitic effectiveness in infants, rat unit for rat unit, of vitamin D administered in the form of cod-liver oil, of percomorph liver oil (a mixture of fish liver oils of high potency), of irradiated cholesterol, of irradiated fresh milk or of irradiated evaporated milk. W.H.R.

459. A Demand for Action. Editorial. Lancet *231*, p. 824, April 3, 1937.

"That underconsumption of a foodstuff so important as milk should exist in a country so eminently suited for milk production, is a matter towards which we cannot remain indifferent," is one of the conclusions of the Ad-

visory Committee on Nutrition of the Ministry of Health of Great Britain, which has recently issued a preliminary report.

The committee states that consumption of milk in Great Britain is now less than one-half the optimum of seven-eighths of a pint per person per day, and they "deplore the fact that, while the volume of milk offered for sale is growing and there is a substantial surplus which it is beyond the capacity of the liquid milk market to absorb, there should be at the same time a severe deficiency of milk in large sections of the population." J.A.T.

460. The Value of Vitamin D Milks to the Consumer. JOHN W. M. BUNKER AND ROBERT S. HARRIS, 23rd Ann. Report of Intern. Assoc. Dairy and Milk Inspectors. p. 139, Oct. 1934.

This paper gives a very comprehensive review of literature on the subject of vitamin D milks.

A summary of clinical reports on vitamin D milks to October, 1934, is included.

The author concludes as follows: (1) That vitamin D milk has proved useful in the diets of infants and their mothers.

(2) That its use has apparently not had any undesirable effects.

(3) The exact antirachitic potency of such milks, which would be most desirable, has not as yet been determined.

(4) It has not yet been determined just what value vitamin D milk would have over ordinary milk for normal adults. L.H.B.

461. Nutritive Value of Chocolate Flavored Milk. W. S. MUELLER AND W. S. RITCHIE, Dept. of Dairy Industry and Dept. of Chemistry, Mass. State College, Amherst, Mass. JOURNAL OF DAIRY SCIENCE *20*, 6, p. 359, June 1937.

When the diet of rats contained more than 2½ per cent cocoa there was a definite retardation of the growth rate. A.C.D.

ICE CREAM

462. The Relationship Between Temperature and Overrun in the Whipping of Ice Cream Mixes. ALAN LEIGHTON AND ABRAHAM LEVITON, Div. of Res. Lab., Bureau of Dairy Ind., U. S. Dept. of Agr., Washington, D. C. JOURNAL OF DAIRY SCIENCE *20*, 6, p. 371, June 1937.

An algebraic equation was derived to show the relation between overrun and drawing temperature of ice cream. A.C.D.

Other abstracts of interest are numbers 436, 437, 439, 442, 444, 446, 452, 453, 455, 457, 458, 460, 461, 463, 465, 466, 467, 468, 472, 473, 475, 476, and 477.

MILK

463. A New Method in Figuring Standardization of Cream and Milk. HANS EDEL, Gehl's Guernsey Farms, Milwaukee, Wis. Milk Dealer *26*, 8, p. 40, May 1937.

The author presents a method in figuring standardization of cream and milk by the use of charts. Advantages claimed for the method other than that it serves calculations are that the results are obtained in gallons and that the weights per gallon for the different percentages of fat in the cream are automatically taken into consideration. C.J.B.

464. One Hundred Per Cent Paper Container Operation. Milk Dealer *26*, 8, p. 42, May 1937.

A brief description is given of the Risdon Plant of Detroit which delivers wholesale only and operates 100 per cent with paper containers. C.J.B.

465. Effecting Sterilization by Radiation. A. R. DENNINGTON, (Westinghouse Lamp Co., Bloomfield, N. Y.) Can. Dairy and Ice Cream J. *16*, 6, p. 32, June 1937.

A discussion is given of a wide field for possible application of the use of short wave lengths for sterilization. It is reported that the experimental radiation of milk in a thin film for 40 seconds at a temperature of 35° F. gave about 98 per cent sterility without changing its flavor. J.C.H.

466. Report of Committee on Dairy and Milk Plant Equipment. WALTER D. TIEDEMAN, 23rd Ann. Report of Intern. Assoc. of Dairy and Milk Inspectors, Oct. 1934, p. 23.

This report gives a draft of tentative specifications for outlet valves to pasteurizers or holders, and for inlet connections to pasteurizers or holders. L.H.B.

467. Official Control of Pasteurization in Massachusetts. HERMAN C. LYTHGOE, 23rd Ann. Report of Intern. Assoc. of Dairy and Milk Inspectors, p. 81, Oct. 1934.

A report of the quality of the milk in Massachusetts during 1933 and 1934 both before and after pasteurizing is given.

A summary of violations found on inspection and of court cases are included. L.H.B.

468. Pasteurization and the Courts. JAMES A. TOBEY, 23rd Ann. Report of Intern. Assoc. of Dairy and Milk Inspectors, p. 109, Oct. 1934.

A review of court cases concerning pasteurization.

From the decisions cited it may be concluded that the judiciary realize that pasteurization is a reasonable measure required to aid in the insurance of a safe milk supply. L.H.B.

469. Certified Milk—Pasteurized. RICHARD S. EUSTIS, 23rd Ann. Report of Intern. Assoc. of Dairy and Milk Inspectors, p. 121, Oct. 1934.

Certified milk pasteurized was first recognized in Boston, Massachusetts, in 1928. During that year about 9 per cent of the total sales of certified milk was pasteurized. During the first half of 1934, 60 per cent of the total sales of certified milk was pasteurized.

Pasteurization of the certified milk has resulted in no change in flavor but the bacteria counts have been lowered.

During the five year period 92 per cent of the bacteria counts on the pasteurized certified milk were 100 or under.

The milk is pasteurized at the farm under supervision of the milk commission.

Vitamin D certified milk was undertaken in 1932 by encouraging the producers to feed irradiated yeast. The increase in the total sales of certified milk both raw and pasteurized since 1933 are attributed to the increasing demand for vitamin D milk. L.H.B.

470. Milk Sanitation in European Countries. ROBERT S. BREED, 23rd Ann. Report of Intern. Assoc. Dairy and Milk Inspectors. p. 164, Oct. 1934.

Improvements in milk sanitation are taking place in all European countries. The greatest advances in milk sanitation are occurring in Great Britain and Italy. The custom of boiling milk in the home is practiced almost universally on the continent so that there has been little development in modern pasteurizing plants. L.H.B.

471. Body of Cultured Cream. E. S. GUTHRIE, Cornell Univ., Agr. Exp. Sta., Ithaca, N. Y. Bul. 666, Feb. 1937.

Pasteurization temperatures near 165° F., with a holding period of 30 minutes, gave the smoothest, driest, and most viscous body of cultured cream.

Homogenization pressures of approximately 3000 pounds to the square inch resulted in the smoothest, driest, and most viscous body. The homogenization temperatures that gave the best results ranged from 165° F. down to 145°, depending on the desired thickness of body. Rehomogenizing at pasteurizing temperatures, or a few degrees below, increased the firmness, dryness, and viscosity of the body.

The raising of the milk-solids-not-fat, by the addition of skimmilk powder, distinctly increased the viscosity and did not affect the texture of the body.

The use of rennet makes a firmer and more viscous body than is obtained when rennet is not employed. Its adaptability is limited by regulations of boards of health.

Gelatin produced a body that lost moisture, which is very undesirable. The viscosity was noticeably increased; the texture, however, was lumpy.

A ropy starter had little effect on the viscosity of the final product.

Increased milkfat above 18 per cent resulted in decreased viscosity.

A maintained ratio of solids-not-fat to fat in cream testing from 18 to 24 per cent of milkfat, gave a body of uniform viscosity. E.S.G.

472. The Effect of Homogenization at Different Temperatures on Some of the Physical Properties of Milk and Cream. RANDALL WHITAKER AND L. D. HILKER, Res. Lab. of Sealtest System Lab., Baltimore, Maryland. JOURNAL OF DAIRY SCIENCE *20*, 5, p. 281, May 1937.

It was found that butterfat in milk or cream must be liquid to obtain proper homogenization. A.C.D.

473. The Effect of Fat Content on Oxidized Flavor in Milk and Cream. CHAS. T. ROLAND AND H. A. TREBLER, Sealtest, Inc., Baltimore, Maryland. JOURNAL OF DAIRY SCIENCE *20*, 6, p. 345, June 1937.

The sensitivity of milk to develop oxidized flavor increased with increased fat content. A.C.D.

474. Mastitis Detection and Control. RALPH B. LITTLE, The Rockefeller Institute for Medical Research, Princeton, N. J. Conven. Intern. Assoc. Milk Dealers, Production Section, p. 71, 1936.

Both acute and chronic types of mastitis can be produced experimentally in the udders of first half heifers by conveying small numbers of streptococci beyond the meatus of the teat. With the short chain streptococci the infection is acute. With the long chain varieties of nonhemolytic streptococci the disease process is much milder.

In the control of mastitis the laboratory examination of the milk is superior to any other single method. However, for the successful operation of any control measure the dairyman should have the full cooperation of his veterinarian. E.F.G.

475. The Vitamin C Content of Cow's Milk. S. K. KON AND M. B. WATSON, Nat'l Inst. for Research in Dairying, Univ. of Reading, England. Biochem J. *31*, p. 223, 1937.

Vitamin C may be present in two forms, as ascorbic acid and as dehydroascorbic acid. Both are biologically active, but the latter does not react with indophenol reagent unless treated with H_2S, is labile and undergoes spontaneous decomposition associated with loss of biological activity. Under the action of light the reduced form of ascorbic acid present in milk is reversibly oxidized to dehydroascorbic acid. This paper deals with the state in which vitamin C is secreted by the cow and presents estimations of the vitamin C content of herd milk at different times of the year under south England conditions.

The healthy mammary gland of the cow secretes milk, the vitamin C of which is only in the reduced form of ascorbic acid. No reversibly oxidized ascorbic acid is found in milk as it leaves the udder. The season of the year and the nutrition of the cows have no effect on the vitamin C content of herd milk. The average figures for herd milk (passed over a surface cooler) from 26 Shorthorn and 3 Guernsey cows is 2.01 mg. of reduced and 2.37 mg. of total ascorbic acid per 100 ml. during a stall feeding period, and 2.06 and 2.35 mg./100 ml., respectively, on early pasture feeding. The vitamin C content of colostrum is only slightly greater than that of milk. K.G.W.

476. **The Source of Vitamin D in Summer Milk.** JOHN EDWARD CAMPION, KATHLEEN MARY HENRY, S. K. KON, AND JAMES MACKINTOSH, Nat'l Inst. for Research in Dairying, Univ. of Reading, England. Biochem. J. *31*, p. 81, 1937.

Eight Shorthorn cows were divided into four groups of two each and placed on the following treatments: Group 1 kept indoors on winter ration which included 5 lb. of hay daily per cow; Group 2 kept outdoors exposed to sun and sky-shine, given same ration as Group 1; Group 3 out on pasture; Group 4 kept indoors but given 1 cwt. daily per cow of freshly cut grass, and the same summer maintenance and production ration as given Group 3. The experiment lasted from May 1 to June 30, 1936. The vitamin D in the fat was determined by prophylactic bone ash tests. The butterfat from cows outdoors and indoors on the summer ration had potencies of 0.46 and 0.15 I.U. per gram, respectively (ratio of potencies 3:1), while the figures for similar butters produced on the winter ration were 0.88 and 0.27 I.U. per gram, respectively (ratio of potencies 3:1). The average total amount of vitamin D secreted daily by a cow was as follows: Group 1, 110 I.U.; Group 2, 313 I.U.; Group 3, 252 I.U.; Group 4, 52 I.U. The yields per kg. of milk were 8.3, 26.0, 17.0 and 5.3, respectively. The cows (whether fed outdoors or indoors) on the winter ration which contained hay secreted more vitamin D than the corresponding cows on summer feed consuming a plentiful supply of fresh grass. It is concluded from the experiments that the direct exposure of the cow to sun and sky-shine contribute all, and the pasture none, of the increase in the vitamin D potency of milk which takes place in summer. K.G.W.

477. **A Comparison of the Vitamin D Contents of Guernsey and Shorthorn Butter (Milk).** STANISLAW K. KON AND KATHLEEN M. HENRY, Nat'l Inst. for Research in Dairying, Univ. of Reading, England. Biochem. J. *30*, p. 776, 1936.

The vitamin D contents of Guernsey and Shorthorn butter fats produced on pasture under similar conditions of feeding and management were compared. The Guernsey butterfat was found to contain 0.35, the Shorthorn

0.28 I.U. of vitamin D per gram, as determined by protective technique and bone ash calcification. The difference is not statistically significant.

K.G.W.

Other Abstracts of interest are numbers 436, 437, 438, 439, 440, 441, 442, 444, 451, 452, 453, 454, 455, 457, 458, 459, 460, and 461.

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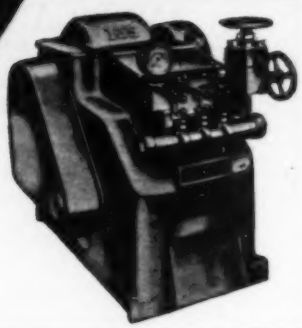

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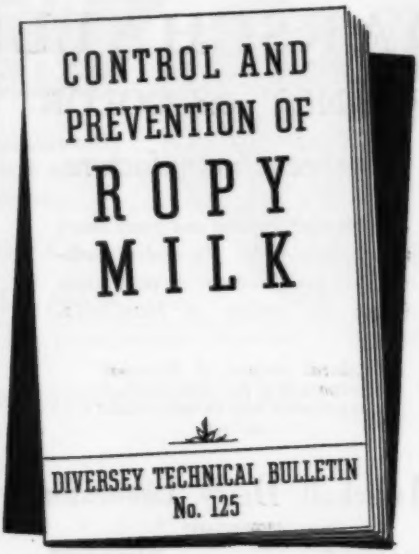

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